Towards elective single embryo transfer: investigating approaches for improving accessibility and efficacy of *in vitro* fertilisation treatment and their implications for patients.

By

Simon Phillips M.Sc.

A portfolio of research submitted in partial fulfillment of the award of the degree of Doctor of Philosophy by Publication

School of Pharmacy and Biomedical Sciences
Faculty of Science
University of Portsmouth
Abstract

This study investigates different modalities for achieving elective single embryo transfer in assisted reproductive technologies (ART) and developing protocols and policies that both permit maximum access to care as well as improving safety of ART by limiting the number of embryos that are transferred.

The study includes 16 peer-reviewed journal articles and 1 book chapter published since 2001 and covering my career to date while working in two university affiliated IVF centres: McGill University and the University of Montreal, Canada. It includes research on:

• **Improving the clinical application of modified natural cycle IVF**

A total of 134 MNCIVF cycles were compared with 370 IVF cycles using COS; in patients under the age of 35 years old the clinical pregnancy rate was 27% versus 47% however in patients older than 35 years old the benefits of MNCIVF did not support the poorer prognostic with a clinical pregnancy rate of only 8%.

The addition of a NSAID, Indomethacin, further optimized the MNCIVF protocol by decreasing the premature ovulation rate from 16% to 6%, this in turn increased the potential of obtaining the oocyte at retrieval from 64% to 76%.

The combination of MNCIVF and surgical sperm retrieval was demonstrated as a viable option in a case report describing a couple who did not want any risk of multiple pregnancy and the least invasive treatment possible to obtain their pregnancies. This treatment was confirmed with a study comparing 81 MNCIVF using ejaculated spermatozoa with 44 MNCIVF using surgically retrieved spermatozoa. There were no differences in the clinical pregnancy rate (31.0% versus 35.1%), however the chance of having an embryo transfer increased in the surgical group (65.9% versus 45.7%). The age of the male partner in the surgical group was older (41.5 versus 36.5) perhaps associated with second relationships; a group for whom MNCIVF can be an interesting treatment option.

The use of MNCIVF in young poor responders is suggested as well as an option for egg donation where the donor wants to avoid gonadotrophins. An analysis of over 1500 MNCIVF was carried out demonstrating a clinical pregnancy rate per embryo transfer of 32.5%. Patient cycles were stratified according to ovarian reserve and in younger patients this had little influence on outcome, however in
the patients over 35 years of age, the clinical pregnancy rate dropped significantly with a poor ovarian reserve (26.3% versus 6.3%)

- Investigating the application of the protocol for *in vitro* maturation of oocytes

One hundred and seven IVM cycles in patients with polycystic ovarian syndrome were case matched with 107 IVF and COS cycles. There was no significant difference in the clinical pregnancy rate (28% versus 41%) although it came close to significance, however there were less oocytes (10.3 versus 14.9), less mature oocytes (7.8 versus 12.0) and less embryos available (5.8 versus 8.6). Importantly there were no cases of OHSS in the IVM group compared to 11% in the IVF with COS group.

A case study describes the use of IVM in combination with PESA and the possibility to successfully obtain and mature oocytes in a PCOS patient and then fertilize those oocytes using spermatozoa retrieved using a simple procedure in an azoospermic partner. Additional more specialized uses of IVM are described including a case report of a young patient with FSH receptor mutation and falling ovarian reserve for whom IVM was used to obtain and mature ten oocytes for fertility preservation following a failed attempt to stimulate her ovaries.

- Studying the implementation of legislation and funding related to ART and the impact on treatment in Quebec, Canada

An initial study after the introduction of funding demonstrated a reduction from 25.6% to 3.7% in the multiple pregnancy rate following IVF, with an increase in the use of eSET from 1.6% to 50% of performed embryo transfers. This was followed by studies showing that although the pregnancy rate in IVF dropped under funding, the cumulative pregnancy rate would correct for this drop using a predictive calculation (29.7% versus 31.9%). This was confirmed with data analysis after the first complete year of funding in one clinic, indicating that the transfer of one fresh embryo followed by one frozen-thawed embryo gave equivalent pregnancy rates to DET (47.1% versus 47.1%).

A financial analysis furthermore supported the fiscal benefits of funding showing that although funding in absolute terms costs the government more, the cost per baby is lower under funding than with a tax rebate system (49,517$ versus 43,362$) considering the costs of high order multiple pregnancies that are significantly reduced under funding with an eSET policy in place.
A final complete analysis of the totality of the funding program after it was shut down, comparing with the year prior to and the year post funding, clearly indicates that the design of funding can influence not only the fiscal burden but also clinical decision making and that this reflects in the results. The cancellation rate increased dramatically (17.0% versus 34.4%) and the live birth rate dropped in all treatment modalities (33.9% versus 23.7%). However, the multiple pregnancy rate dropped from over 25% to under 5% throughout the course of funding and remained under 5% even once funding was withdrawn.

These publications form a tiny part of the enormous journey that ART has taken over the course of the last 40 years and continues to take on a daily basis. Additional research is required in all these and other areas related to IVF and embryology in order to further improve all aspects of ovarian stimulation, embryo culture and selection and implantation knowledge and application.
Acknowledgements

Without the support of my wonderful wife, Annick and our family Meghane, Oliver, Abigail and Joshua, none of this would have been possible.

I would like to thank my parents and sister who have always believed in me.

Over the years, I have been lucky enough to have worked with a number of incredible people from whom I have learnt a great deal and continue to learn every day. This research is the result of collaboration with many of these individuals and would not have been possible without them: REI consultants, REI fellows, and scientist colleagues; I am grateful to them all for the opportunity to have worked with them.
Declaration

I declare that whilst studying for the degree of Doctor of Philosophy by Publication at the University of Portsmouth I have not been registered for any other award at another university. Furthermore the work undertaken for this degree has not been submitted elsewhere for any other award. The published works contained in this submission are my work and where the work of others is referenced it has been duly acknowledged in the text.

Simon Phillips
June 2018
Ethics Declaration

For all studies and papers included in this thesis, Ethics approval was obtained from the Institutional Review Board at either McGill University Health Centre (MUHC) or the University of Montreal Health Centre (CHUM) as applicable or in the case of retrospective data analyses approval was obtained from the local Scientific Review Board as appropriate.

Ethics approval was not required for the studies using data obtained from the CARTR registry according to the Canadian Tri-council Policy statement on Ethical Conduct for Research involving Humans since data was obtained from a centralized anonymized databank.
# Contents

**Abstract**  

**Acknowledgements**  

**Declaration**  

**Ethics Declaration**  

**Contents**  

**List of Abbreviations**  

**Chapter One – Introduction**  

**Chapter Two – Application of Modified Natural Cycle IVF**  

**Chapter Three – In Vitro Maturation of Oocytes**  

**Chapter Four – Government Funding of IVF**  

**Chapter Five – Conclusions**  

**References**  

**Additional Published Works by the Author**

- **Scientific Articles**
- **Published Abstracts**

**Appendix 1 – Controlled Natural Cycle IVF: Our Experience in a World of Stimulation**

**Appendix 2 – Natural Cycle IVF: A Question of Semantics**

**Appendix 3 – Spontaneous Ovulation Rate Before Oocyte Retrieval in Modified Natural Cycle IVF With and Without Indomethacin**

**Appendix 4 – Ongoing Pregnancy After ICSI of Frozen-Thawed PESA-Retrieved Spermatozoa and IVF in a Controlled Natural Cycle**

**Appendix 5 – Surgically Retrieved Spermatozoa Versus Ejaculated Spermatozoa in Modified Natural Cycle IVF-ICSI Cycles**

**Appendix 6 – Modified Natural Cycle in Vitro Fertilization Should Be Considered as the First Approach in Young Poor Responders**

**Appendix 7 – Successful Pregnancy in an Ovarian Agenesis Patient After Modified Natural Cycle IVF Oocyte Donation**

**Appendix 8 – Ovarian Rejuvenation with Dehydroepiandrosterone Prior to a Modified Natural in Vitro Fertilization Cycle: A New Hope in Premature Ovarian Insufficiency**

**Appendix 9 – Outcomes of 1503 Cycles of Modified Natural Cycle in Vitro Fertilization: A Single-Institution Experience**
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ART</td>
<td>Assisted reproductive technologies</td>
</tr>
<tr>
<td>BORN</td>
<td>Better Outcomes Registry Network</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CARTR</td>
<td>Canadian ART Registry</td>
</tr>
<tr>
<td>CDC</td>
<td>Centre for Disease Control and Prevention</td>
</tr>
<tr>
<td>CFAS</td>
<td>Canadian Fertility and Andrology Society</td>
</tr>
<tr>
<td>COS</td>
<td>Controlled ovarian stimulation</td>
</tr>
<tr>
<td>DET</td>
<td>Double embryo transfer</td>
</tr>
<tr>
<td>DHEA</td>
<td>Dehydroepiandrosteronedione</td>
</tr>
<tr>
<td>eSET</td>
<td>Elective single embryo transfer</td>
</tr>
<tr>
<td>FET</td>
<td>Frozen embryo transfer</td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
</tr>
<tr>
<td>hCG</td>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>hMG</td>
<td>Human menopausal gonadotropin</td>
</tr>
<tr>
<td>ICSI</td>
<td>Intracytoplasmic sperm injection</td>
</tr>
<tr>
<td>IVF</td>
<td><em>In vitro</em> fertilisation</td>
</tr>
<tr>
<td>IVM</td>
<td><em>In vitro</em> maturation</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing hormone</td>
</tr>
<tr>
<td>MNCIVF</td>
<td>Modified natural cycle in vitro fertilisation</td>
</tr>
<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OHSS</td>
<td>Ovarian hyper-stimulation syndrome</td>
</tr>
<tr>
<td>PCOS</td>
<td>Polycystic ovarian syndrome</td>
</tr>
<tr>
<td>PESA</td>
<td>Percutaneous sperm aspiration</td>
</tr>
<tr>
<td>PGT-A</td>
<td>Pre-implantation genetic testing for aneuploidy</td>
</tr>
</tbody>
</table>
Chapter 1 – Introduction

The birth in 1978 of Louise Brown signaled one of the greatest developments of reproductive medicine: the clinical application of many years of basic research in human reproduction and the beginning of numerous advances in the field (Steptoe and Edwards, 1978). This first *in vitro* fertilisation (IVF) birth was the result of obtaining an oocyte during the natural menstrual cycle of the patient. The team of Edwards and Steptoe had previously worked unsuccessfully to perform IVF in combination with ovarian stimulation before returning to the natural cycle and it was not until 1981 that the first IVF baby was born following ovarian stimulation and the recruitment of multiple follicles (Trounson *et al*, 1981). Over the course of the next fifteen years a number of important ‘firsts’ were associated with IVF treatment: birth after embryo cryopreservation (Trounson *et al*, 1983), the genetic analysis and selection of embryos for x-linked and monogenic disease (Handyside *et al*, 1990, 1992), intracytoplasmic sperm injection (ICSI): the microinjection of sperm into the egg to achieve fertilisation in cases with compromised sperm parameters (Palermo *et al*, 1992) and the application of surgically retrieved testicular spermatozoa in fertilisation using ICSI (Silber *et al*, 1994).

For many reasons it was believed that IVF in combination with controlled ovarian stimulation (COS) would be the most effective use of the treatment and from 1981 the use of IVF with COS became the standard. Controlled ovarian stimulation was achieved initially with clomiphene citrate, a selective estrogen receptor modulator (Lopata *et al*, 1980) and then using human menopausal gonadotropin (hMG) (Trounson *et al*, 1981) along with gonadotropin-releasing hormone (GnRH) agonists (Fleming *et al*, 1982). IVF with COS in combination with embryo cryopreservation offered patients the ability to have multiple pregnancy attempts from one ovarian stimulation cycle and it also allowed for the transfer of multiple embryos which increased the chance of pregnancy. However, this option to transfer more than one embryo at a time also introduced the side-effect that would become arguably the biggest issue associated with *in vitro* fertilisation for many years; that is the increased risk of multiple pregnancies. The first multiple pregnancies resulting from IVF were reported in 1983 in Australia (Speirs *et al*, 1983). In North America by the start of the new millennium the multiple pregnancy rates from IVF were reported as 33% to 54% of all live births (Reports available on Centre for Disease Control and Prevention (www.cdc.gov) and Canadian Fertility and Andrology Society (www.cfas.ca) websites).
Other than the availability of only one oocyte the major driving force away from using natural cycle for IVF was the inability to control the cycle. The first IVF cycles were performed by monitoring the natural luteinizing hormone (LH) surge of the patient and carrying out the oocyte retrieval based on this timing. The result of this approach is a requirement to have the operating room and laboratory available at all times of the day and night and an extremely high risk of premature ovulation and loss of the oocyte.

The development of GnRH antagonists opened the opportunity to revisit the use of natural cycle IVF (Frydman et al, 1991). Unlike GnRH agonists that cause indirect suppression of the pituitary, the administration of GnRH antagonist results in immediate, dose-related inhibition of gonadotropin release by competing for GnRH receptors in the pituitary. This immediate short-term effect removes the need for pre-cycle down-regulation of the ovary and permits pre-ovulatory application. Despite being proposed in the early 1990s, it took over 10 years before GnRH antagonists started to be used regularly rather than GnRH agonist protocols as the standard for IVF with COS.

In 2004 we started offering a controlled natural IVF cycle to patients at our clinic. In 2007, I published a paper describing a series of 134 controlled natural IVF cycles comparing them with 370 IVF cycles using COS in patients under-35 years of age (Phillips et al, 2007). The clinical pregnancy rate in these controlled natural cycles was 27% with no multiple pregnancies compared to a clinical pregnancy rate of 47% in the IVF with COS cycles but at the cost of a 37% multiple pregnancy rate. In addition, in this paper, I analysed the use of controlled natural cycle IVF in 108 patients over the age of 35 who requested controlled natural cycle IVF despite counselling to the contrary; these patients had a clinical pregnancy rate of only 8% as compared to 240 cycles of over-35-year-old patients with IVF and COS who had a clinical pregnancy rate of 37% with a multiple pregnancy rate of 36%.
For the under 35-year-old group of patients undergoing controlled natural cycle IVF, the 134 cycles were performed for 70 patients, with a clinical pregnancy rate per patient of 28.5%. In this paper I concluded that although the clinical pregnancy rate was lower for the controlled natural cycle IVF group, the benefits of a simpler protocol, fewer injections and medication, the ability to carry out an IVF month after month like intrauterine insemination cycles and no risk of multiple pregnancy was an interesting option for some patients. Based on this study patients were counselled to move to IVF with COS after three failed attempts of controlled natural cycle IVF since over 90% of patients who got pregnant did so on cycle one or two. Patients over the age of 35 were also counselled against controlled natural cycle IVF since the pregnancy rate in this group was particularly low.

The application of hMG alongside GnRH antagonist in our protocol for controlled natural cycle certainly created some debate as to how ‘natural’ the protocol was. As journal referee after referee asked for clarification I published a letter outlining the basis for this use and why the cycle should still be considered ‘natural’ (Phillips et al, 2007). Kettel had demonstrated in 1991 that the application of a GnRH antagonist Nal-Glu, concomitantly with gonadotropin suppression, causes significant drops in estradiol levels leading to functional arrest in the dominant follicle (Kettel et al, 1991). This effect can be seen in temporarily plateauing estradiol levels in COS; however, the impact is more critical in natural cycles with only one follicle available for oocyte retrieval: the small dose of hMG given concurrently with the start of GnRH antagonist acts simply to limit the estradiol drop and support the follicle. We proposed the term controlled natural cycle IVF in our letter, and various other terms were used at the time. Consensus determined that the protocol is now referred to as ‘modified natural cycle IVF’ (Nargund et al, 2007).

Now that control over the cycle could be assured there were still a disproportionately high number of cycles that ended with no embryo transfer. The use of ICSI could maximise the chance of fertilisation, but premature ovulation despite GnRH antagonist and failure to collect the oocyte at retrieval were still problematic. Follicle flushing and the use of 0.22 µm filters at egg collection, as used in in vitro maturation (to be discussed later), helped to maximise oocyte retrieval, however, the rate of premature ovulation was still high.

Non-steroidal anti-inflammatory drugs (NSAIDs) are known to affect ovulation likely through disruption of cyclooxygenase 2 (COX-2) which is the rate-limiting enzyme in prostaglandin production (Gaytan et al, 2006). A case report published in 1996 demonstrated that indomethacin, a NSAID (marketed in Canada as Indocid by Merck Frosst) could be used to delay ovulation for up to seven days. Patients from a community where the male partners were often absent for work
commitments were given the NSAID to delay ovulation in intrauterine insemination cycles until the male partner was available to produce a semen sample (Nargund and Wei, 1996).

In 2008, I authored a publication with a series of modified natural cycles looking at the addition of indomethacin, demonstrating that the use of the NSAID in addition to our standard modified natural cycle protocol reduced the premature ovulation rate from 16% to 6% (OR 3.8 95%, CI 1.2-12.3) and increased the oocyte retrieval rate from 64% to 76% (p < 0.04) (Kadoch et al, 2008). The use of indomethacin had no impact on the pregnancy rate although there was a clinically interesting increase for those patients who took the drug (14% versus 21% p = 0.13).

This approach was confirmed by a Japanese group who also used NSAID in modified natural cycle to reduce the risk of premature ovulation (Kawachiya et al, 2012).

These papers demonstrated that a modified natural cycle IVF could be offered to patients with the associated benefits of an easier process for the patient, less costly, no risk of ovarian hyper-stimulation syndrome (OHSS), the possibility to perform cycles month after month and almost no risk of multiple pregnancy. Although clinical pregnancy rates were lower using modified natural cycle, ranging in the literature between 10% and 50%, for those patients opting for this protocol the benefits outweighed this limitation.

Our work on modified natural cycle IVF was part of a movement at the time promoting a gentler approach towards IVF. Other researchers were also working with modified natural cycles as well as mild stimulation protocols for IVF. These different approaches were highlighted in a review editorial by Edwards in 2007 and underlined by the creation of a society directed entirely at the promotion of more physiological and mild IVF protocols; The International Society for Mild Approaches in Assisted Reproduction (ISMAAR) (Nargund and Frydman, 2007).

In their 2007 paper, ISMAAR proposed terminology for ovarian stimulation protocols including the now accepted definition of modified natural cycle IVF. In addition, natural cycle IVF was defined as IVF carried out in a spontaneous menstrual cycle without the administration of any medication at any time during the cycle differentiating it from MNCIVF. In contrast mild IVF was defined as the application of FSH or HMG at lower doses than used in standard COS or for a shorter duration or when oral compounds are used in combination with gonadotropins. The aim of mild IVF is to collect between 2 and 7 oocytes. Studies have suggested that mild IVF reduces aneuploidy (Baart et al, 2007); in this randomized study the absolute number of euploid embryos was the same in the mild IVF group and the IVF with COS group, however proportionally the rate of aneuploidy was lower in the mild
IVF group. It has also been suggested that the use of mild IVF regimes can improve oocyte quality over IVF with COS in certain patients (Jamal et al, 2009). More recently a review of the use of mild IVF was published in 2009 (Verberg et al, 2009) as well as a debate on the subject (Fauser et al, 2010). These articles reference the fact that to date there are only a limited number of randomized control trial studies (RCT) comparing mild IVF with IVF using COS. However, some data is presented demonstrating reduced costs, safer treatment for patients and answering concerns around the milder IVF protocols in terms of potentially having lower cumulative pregnancy rates if fewer embryos are available: the data suggests that the overall number of available good quality embryos is the same thereby countering this argument. Both articles speak to the fact that mild IVF protocols have not been widely incorporated and suggest clinical resistance and patient characteristics (advanced maternal age) as primary reasons for this. Further research to demonstrate benefits are proposed going forward to underline the potential increased use of milder IVF protocols in ART.
Chapter 2 – Applications of modified natural cycle IVF

Having improved the efficacy of the modified natural cycle for IVF (MNCIVF), it was clear that the benefits of this treatment were not apparent for all groups of patients: for example, my original study suggested significantly reduced pregnancy rates in patients using modified natural cycle IVF over 35 years of age. Other studies looking at age related outcomes in MNCIVF confirmed this pattern; Tomazevic looked at patients less than and greater than 39 years of age and saw significant differences in the outcomes measures. Live birth rates were 55% versus 29% per embryo transfer in his study of 397 cycles (Tomazevic et al, 2007). Another study of 947 MNCIVF cycles over 5 years confirmed the importance of age when three age groups were compared (≤ 35, 36-39 and ≥ 40), the authors reported clinical pregnancy rates that reduced by age group (10.6% versus 7.6% versus 3% respectively) (Gonzalez-Foruria et al, 2016).

However, the ease of the protocol, reduced costs, minimal application of medication and no risk of multiple pregnancy are very appealing to certain patients. In 2005, I authored a case report for the use of MNCIVF in combination with percutaneous sperm aspiration (PESA) a relatively non-invasive and simple urological procedure to obtain sperm in men with an obstructive azoospermia (Kadoch et al, 2005). In this case the couple had two children together; the male partner had undergone a vasectomy believing that their family was complete. When the couple decided that they wanted another child he underwent a failed vasovasostomy procedure. Since IVF presented the only option at this point but the couple were adamant that they did not want any risk of twins and wanted the simplest treatment available to them, MNCIVF fulfilled these requirements. Since second relationships where the male partner has undergone a vasectomy previously and where one or both of the couple already have children is a sub-group of patients presenting for care at IVF clinics, MNCIVF presents an alternative that can be offered to these patients.

The application of surgical sperm retrieval in combination with MNCIVF was confirmed by a study I authored in 2012 which compared the outcomes of MNCIVF using ejaculated sperm with surgically retrieved sperm (Jamal et al, 2012). In this study no significant difference was seen in the clinical pregnancy rate for those cycles using surgically retrieved sperm when compared to ejaculated sperm as the source for ICSI (35.1% versus 31.0%, p = 0.71). No differences were seen in the female related parameters in the two groups although the men in the surgically retrieved sperm arm were significantly older; (41.5% versus 36.5%, p = 0.001) which may reflect a group as mentioned earlier; that is men in second relationships who have previously undergone vasectomy.
Another group that could benefit from MNCIVF are young patients who respond poorly to COS. In these cases, oftentimes high doses of gonadotropins are used in order to try to elicit a response from the ovaries, but despite the high doses perhaps only one or two follicles can be recruited. In this case, the use of MNCIVF can be a more physiologically appropriate, cost effective, and easier proposition for the patient. In addition, it avoids super-physiological doses of gonadotropins which may have a deleterious effect on the endometrium. This was proposed in two publications in 2009 demonstrating important differences in the gene expression profiles from the endometrium in natural cycles when compared to COS cycles in the same patients (Haouzi et al., 2009). Potential effects directly on the oocytes themselves have also been suggested in the mouse model (Edgar et al., 1987). We recommended the approach of using MNCIVF as a first line treatment for poor responder younger aged patients in a published letter to Fertility and Sterility in 2011 (Kadoch et al., 2011) outlining the benefits of MNCIVF and reduced risks to patients in particular to those who require high doses of gonadotropins for ovarian stimulation. This concept was supported by published data demonstrating a statistically significantly improvement in implantation rate using MNCIVF in poor responders in a randomised trial comparing to IVF with COS (14.9% versus 5.5%, p = 0.05) (Morgia et al., 2004).

I also authored published case reports demonstrating the potential for the use of MNCIVF for specific patient cases further confirming the benefit of having MNCIVF as an additional protocol option for fertility clinics to offer. A 38-year-old patient with the rare condition of ovarian agenesis required oocyte donation to treat her infertility. Her sister offered to be the oocyte donor and since the patient and her sister wanted to minimise the impact on the donor and her life, it was proposed to use MNCIVF for the egg donation cycle. A successful cycle resulted in a healthy live birth (Kadoch et al., 2009).

In another case report, a 33-year-old patient with idiopathic primary ovarian insufficiency and a familial history of premature menopause was pre-treated with dehydroepiandrosterone (DHEA) to lower endogenous FSH levels and permit the patient to undergo a MNCIVF. Once again, a successful cycle resulted in a healthy live birth (Lehmann et al., 2014).

Of course, not everyone was convinced about the benefits of MNCIVF and mild stimulation IVF protocols; a 2011 commentary in the journal, Reproductive Biomedicine Online questioned the application of these approaches in critiquing a specific publication but negatively referencing multiple publications on mild stimulation IVF, ISMAAR and MNCIVF including my 2007 paper (Gleicher et al., 2011). The authors’ principal arguments against these approaches hinged firstly on a lack of prospective randomised data as well as the importance of ensuring that patients are fully informed as to potential negative aspects of milder protocols; primarily that cumulative pregnancy rates are also
important and MNCIVF does not offer this possibility whilst mild stimulation significantly reduces the possibility. Clearly prospective randomised trials are the gold standard which are often not performed in the ART field for various reasons, and clear precise informed consent is an absolute requirement for any treatment ensuring that the patient understands the risks and negative aspects of any proposed treatment as well as the potential benefits. In our experience, at least at the time in question, many patients, but certainly not all, found the less invasive nature of MNCIVF to be a very attractive option for their fertility care. Of course, the patient input can be difficult to quantify scientifically and present in a published format, hence limiting the impact of this aspect on the arguments for or against a particular treatment. The use of MNCIVF in developing countries is an example of this; a study from Africa reviewed the reasons why so many patients cancelled their IVF cycles demonstrating that over 75% of cancellations were for financial reasons and these patients when given the choice between lower costs and simpler treatments such as MNCIVF would prefer these options even considering lower pregnancy rates (Shahin, 2007).

In 2015, I authored the largest published series of MNCIVF cycles, analysing 1503 cycles performed in 782 patients over a period of six and a half years (Shaulov et al., 2015). Once again we stratified patients based on age (≤ 35 and ≥ 36) and in addition within the age groups by ovarian response according to the 2011 Bologna criteria (Ferraretti et al., 2011). Once again we confirmed that age is the major factor for the success of MNCIVF with higher cancellation rates in the older group of patients (23.8% versus 17.4%, p = 0.02) and lower clinical pregnancy rates per embryo transfer (23.5% versus 34.5%, p = 0.02). When analysing by ovarian response, in the younger age group the clinical pregnancy rate was not affected by whether the patient had normal or poor ovarian reserve (35% versus 30%, p = 0.82) whereas in the older group the impact was more important (26% versus 6%, p = 0.06). This confirmed our previous proposition to offer MNCIVF to younger patients with anticipated poor ovarian response. In addition, we analysed the results per attempt number from first cycle to fifth MNCIVF attempt. Obviously, the number of cycles carried out reduced over time so 782 first attempts dropped to only 21 fifth cycles, however, the data indicated reasonably stable clinical pregnancy rates per embryo transfer (34.5%, 34.7%, 23.5%, 21.6%, 42.9%).

The major limitation of this series was the retrospective nature of the study and the fact that patients who did not continue to undergo MNCIVF were not followed. These patients could have moved to other centres, converted to IVF with COS or achieved spontaneous pregnancy but these data were not recorded.
The benefits of MNCIVF have been somewhat reduced by the use of GnRH antagonist in combination with GnRH agonist trigger and freeze-all embryo strategies which can almost eliminate the risk of OHSS. Improved cryopreservation techniques and the use of vitrification in particular means that transferring a cryopreserved embryo offers the same potential as a fresh embryo transfer and therefore the importance of cumulative pregnancy rates becomes even more significant. However, our series of publications on MNCIVF demonstrates that it has a place in the arsenal of protocols that can be employed to treat patients and should be kept in mind for specific patients.
Chapter 3 – *In vitro* maturation of oocytes

As already mentioned; other than multiple pregnancy, another major side-effect of IVF using COS is the risk of ovarian hyper-stimulation syndrome (OHSS). OHSS is an iatrogenic complication of COS, and although most OHSS is mild, when severe it can be associated with serious outcomes including reported cases of death. OHSS occurs in patients undergoing COS after an LH surge or exposure to human chorionic gonadotropin (hCG); used to trigger oocyte maturation prior to oocyte retrieval, or secondary OHSS in a pregnant patient following embryo transfer. The primary physiologic change that OHSS presents is a fluid movement from intravascular to third space areas, resulting in abdominal bloating, large ovaries, and in serious cases ascites, hemoconcentration, acute respiratory distress and thromboembolism. (Shmorgun and Claman, 2017)

Polycystic ovaries are one of the risk factors for OHSS since these patients usually present with high antral follicle counts and often over respond to gonadotropin application resulting in multi-follicular response with extremely high levels of estradiol and associated increased risk of OHSS when triggered with hCG. Furthermore, the large number of oocytes retrieved in these patients often results in lower than average rates of maturation and thus increased numbers of immature oocytes which cannot fertilize.

*In vitro* maturation of oocytes shares some of the benefits of MNCIVF, namely less medication, cheaper costs to the patient and greater patient convenience. In 2001 I authored a case report announcing the first case of *in vitro* maturation (IVM) in combination with percutaneous sperm aspiration (PESA) which was indicated due to diagnosis of obstructive azoospermia in the male partner (Abdul-Jalil *et al.*, 2001). The female partner had 35 follicles between 4-6 mm on ultrasound on day 3 of her menstrual cycle indicating a good candidate for IVM. A follow-up ultrasound on day 7 confirmed no dominant follicle and hCG was given since previous work in our clinic had demonstrated the benefits to oocyte retrieval in IVM by giving hCG as used traditionally in an IVF cycle with COS (Chian *et al.*, 2001). Twelve immature oocytes were obtained and cultured for twenty-four hours in specially designed in-house culture media including hMG and maternal serum, resulting in six mature oocytes which underwent ICSI. Four oocytes fertilised normally and three embryos were transferred to the patient on day 2 of embryo development. The patient became pregnant with a clinical twin pregnancy.

IVM demands special consideration at the oocyte retrieval process in terms of both the clinical and laboratory elements. Since small follicles are to be punctured, a special larger gauge needle is required
as is reduced pressure on the aspiration pump. On the laboratory side, immature oocytes do not have expanded cumulus granulosa cells complexes making them very much more difficult to visualise by the embryologist at the retrieval. In order to reduce the risk of missing oocytes at retrieval we added the use of 0.22 µm filters to the procedure and developed a technique of ‘sliding’ to assess oocyte maturity without denudation of the cumulus granulosa cells. In ‘sliding’, oocytes are examined in small volumes of media, tilting the dish in order to flatten the oocyte-cumulus complex, offering a better visual of the oocyte within and permitting an approximation of maturity.

In 2002 I authored a study comparing IVM with IVF and COS for patients with polycystic ovarian syndrome (PCOS): 107 cycles of IVM were case matched with 107 cycles of IVF and COS (Child et al, 2001). Of 1102 oocytes that were retrieved for IVM, 835 matured to metaphase II (76%), fertilisation was achieved in 76% by ICSI as compared to 78% for IVF cycles (no significant difference). The number of retrieved oocytes (10.3 versus 14.9 p<0.01), the number of metaphase II oocytes (7.8 versus 12.0 p < 0.01) and the number of cleaved embryos available (5.8 versus 8.6 p < 0.01) were all lower in the IVM group of patients. Furthermore the number of transferred embryos was lower in the IVF group (3.2 versus 2.7 p < 0.01), the pregnancy rate was not significantly different in the two groups (28% versus 41% p = 0.06) and the clinical pregnancy rate was not significantly different either (23% versus 36% p = 0.05), although both were close to statistical significance. There was a significant difference in the implantation rate (9.5% versus 17.1% p < 0.01) and probably associated with the fact that more embryos were transferred following IVM, the multiple pregnancy rate was higher in IVM although not significantly (41% versus 37%). There was also a significant difference in the incidence of OHSS between IVM and IVF groups (0% versus 11% p < 0.01). The technique succeeded in eliminating OHSS from these high risk patients however; clearly this technique did not achieve the advantage seen later with MNCIVF in removing the risks of multiple pregnancies. The benefits proposed in this study other than elimination of OHSS risk included less costs to the patients: the IVF group took an average of 31 ampoules of follicle stimulating hormone (FSH) during their controlled ovarian stimulation and only one ampoule of hMG was required for the IVM patients, as well as simplification of the cycle from the patients’ point of view with less clinic visits and blood tests requested.

The findings of our study were confirmed in a more recent publication also comparing IVM with IVF using COS in patients diagnosed with PCOS. The researchers found no difference in the clinical pregnancy rates between the groups, however, lower live birth rates were seen in the IVM group (18.8% versus 31%, p = 0.021) although no significant differences were seen in results from subsequent frozen embryo transfer cycles (FET) between IVM and IVF (Walls et al, 2014) Similar
results indicating lower live birth rates but the elimination of OHSS were demonstrated in another study in 2012: live birth rates (44.3% versus 16.5% \( p < 0.001 \)) and development of OHSS (8.2% versus 0% \( p < 0.001 \)) (Gremeau AS et al., 2012).

There have been, however, some concerns raised regarding the use of in vitro maturation. Important epigenetic events in the latter stages of oocyte maturation have caused some researchers to question the safety of the technique. Indeed, the current American Society of Reproductive Medicine (ASRM) guidelines for IVM propose that it should only be used as an experimental technique and patients need to be advised that there is limited evidence related to its safety since relatively few babies have been born as a result of the technique. Some data had demonstrated abnormal methylation in in vitro matured oocytes however it is important to note that the oocytes used in the study were obtained from IVF using COS cycles which had not matured in response to maturation trigger in vivo. These types of oocytes have subsequently been shown to be associated with high levels of DNA damage and poor embryonic development potential and as such are poor study substitutes for in vitro matured oocytes (Coticchio et al., 2015).

As already discussed, hCG used to mimic the natural LH surge and trigger follicle maturation initiates OHSS due to its long half-life and luteotrophic action. The advent of the use of GnRH antagonists in IVF protocols permitting not only protocols such as the aforementioned MNCIVF but also allowed for the use of GnRH agonist application to trigger release of LH and FSH in an acute surge similar to that seen in the normal menstrual cycle. This form of action which was first suggested by a study in 2002 (Fauser et al. 2002) was not proposed specifically for OHSS avoidance until 2006 when a series of randomised controlled studies demonstrated that the use of GnRH agonist to replace hCG as the maturation trigger could effectively eliminate OHSS (Acevedo et al. 2006, Babayof et al. 2006, Engmann et al. 2008) When information from these three studies is combined, in 78 patients triggered with GnRH agonist, no cases of OHSS were reported as compared with 25% OHSS in the group of 78 patients triggered with hCG. This development, which remains as current \textit{practice} today, effectively negated the major benefit of IVM leaving it on the sidelines of standard protocols used in assisted reproduction.

However, it is important to note that it can still be used for specific cases, and similar to MNCIVF should be maintained in the options available for treatment of certain patients. I authored a case report, published in 2017 demonstrating the use of IVM for a very specific case in which other more standard ART protocols did not succeed (Pradervand et al. 2017). A 19-year-old patient presented with a normal antral follicle count and anti-mullerian hormone (AMH) level (1.59 ng/mL) but with primary
amenorrhea and moderately developed secondary sex characteristics, small ovaries and uterus. Her FSH level was surprisingly high at 72.5 IU/L and FSH receptor (FSHR) mutation was suspected. The patient was found to have a rare variant mutation on exon 6 of the FSHR gene. One year later, her AMH dropped to 0.62ng/mL and her antral follicle count similarly reduced to a level associated with ovarian failure. An attempt to provide ovarian stimulation with the goal of fertility preservation was unsuccessful after the patient took 900 IU of recombinant FSH daily for 17 days with no ovarian response at all.

IVM was offered as an alternative treatment; the patient underwent three IVM cycles over the course of several months achieving a total of 10 metaphase II oocytes and 2 metaphase I oocytes which were vitrified for future use. Based on her young age and a total of 10 metaphase II oocytes this patient should have a good chance to conceive in a future IVF cycle using her warmed oocytes (Doyle et al 2016). The normal process for reactivation of the oocyte maturation process explains how in this case immature oocytes could be matured in vitro despite the failure of ovarian response in vivo. The pathway that prevents germinal vesicle breakdown and further oocyte maturation involves signalling between the granulosa cells and the oocytes, that maintains high cyclic adenosine monophosphate (cAMP) within the oocytes. This communication is lost in vivo in response to the natural LH surge; however the physical removal of granulosa cells in vitro mimics the effect resulting in reducing cAMP and triggering the resumption of oocyte maturation. This technique has often been used experimentally in mammalian models to induce oocyte maturation (Jaffe and Norris 2010).

The use of IVM for less common applications has been proposed by other authors including in fertility preservation for oncologic indications such as leukemia, for severe endometriosis as well as in combination with ovarian transposition prior to radiation therapy (Grynberg et al 2013).
Chapter 4 – Government funding of IVF

Despite improving ovarian stimulation protocols and laboratory techniques the drive to replace multiple embryos continued and in 2010 the multiple pregnancy rate was still 29% in Canada (CFAS 2010 report) and 30% in the USA (CDC 2010 report). Furthermore, in the USA, the use of elective single embryo transfer (eSET) was only reported in 10% of patients under the age of 35, and the average number of embryos transferred in this young, normally good prognostic, group of patients was 2.0 (CDC 2010 report).

The use of eSET varies dramatically around the world with several Scandinavian countries leading the way reaching 69.4% eSET rate in Sweden. The major factors that seem to impact the use of eSET are financial, cultural or social and legal. Certain countries have a legal imposition of eSET either in general or for specific cases such as patients less than 35 years of age and this may be associated with public funding as in New Zealand. There are also a number of countries where eSET is the standard even without a legal requirement such as Norway, Denmark and the Netherlands however IVF cycles are funded in these countries (Maheshwari et al, 2011).

In Canada health care is provincially regulated and therefore the different provinces decide whether or not to cover specific treatments and medications. Prior to 2010, the province of Quebec did not include any fertility treatments under the government health insurance although a tax rebate was available for up to 20 000$Can per year of associated costs related to obtaining fertility treatments. In 2010 the government of Quebec introduced a programme of coverage for IVF and I authored a paper describing the effects of the first three months of the programme (Bissonnette et al, 2011).

Assisted reproductive technology (ART) was included under the provincial health plan from August 2010. The policy permitted any woman of reproductive age to have up to three cycles of IVF with COS or up to six attempts of MNCIVF. A cycle was counted at completion of embryo transfer. The cryopreservation of embryos and their subsequent transfer was also included but did not count against the total number of cycles and patients were obliged to use those embryos before carrying out an additional IVF cycle: so, embryo banking, for example, was not possible. The associated medications, required for COS and luteal support, in ART were also placed on the insured drug list for the province.

Alongside the coverage, a law was enacted to limit the number of embryos that could be transferred in any one cycle. The law stated that only one embryo should be transferred however it set hard limits of a maximum of 2 embryos in a patient of 36 years of age or younger and up to three embryos (including no more than two blastocysts) in a patient of 37 years or older. Therefore, the option for multiple
embryo transfer remained available to clinicians and patients but clinics were asked to reduce the multiple pregnancy rate.

I analysed the first three months of all IVF cycles started in Quebec from all the clinics. The average age of patients treated was 37 (range 22-46 years); all clinics applied their own parameters to select ovarian stimulation protocols and day of embryo transfer which ranged from day 2 until day 5. There were 1353 cycles performed resulting in 1103 embryo transfers. Of importance eSET was used in 50% of all embryo transfers and in the age group less than 35 years old, eSET was applied in 79% of embryo transfers. These transfers resulted in 32% clinical pregnancy rate overall and 50% clinical pregnancy rate in the younger group of patients. As a result of these transfers there were a total of 14 twin pregnancies (of which one was in the under 35 age group) and no triplet pregnancies: all the twin pregnancies were the result of double embryo transfer. The multiple pregnancy rate was therefore 3.7%, as compared to 25.6% for the year 2009. We reported this data as indication of the success of the programme since the primary objectives were to encourage access to ART for all patients and to reduce the multiple pregnancy rate: this initial data seemed to support this finding.

Interestingly this publication was not universally supported and in the same edition of the journal a commentary was published by one of the editors presenting the case against government intervention (Gleicher, 2011). In his commentary Gleicher argues that for many patients who wish to have two children, a twin pregnancy is, in fact, a less costly and less risky outcome. He further argues that the transfer of a single embryo reduces the pregnancy rate and therefore fails the patients that it is designed to help. This opinion gained some traction however other authors (Stillman et al, 2013) counter argued the four principle tenants of his argument for twin pregnancies, specifically:

1. **Patient autonomy** – informed consent requires that patients have given all the appropriate counselling prior to treatment and the medical team have a responsibility as the ‘experts’ to guide patients.

2. **DET is more efficient** – there are studies that demonstrate that a double embryo transfer (DET) results in higher pregnancy rates however other studies have shown that the transfer of a single euploid blastocyst results in the same pregnancy rate. Furthermore, the importance of cryopreservation must not be ignored with the potential for obtaining the desired two babies from one COS but through two separate embryo transfers.

3. **Two babies at a time is cheaper** – there are multiple studies indicating that the costs associated with multiple gestation and delivery even excluding the potential long-term care costs are higher than those with singletons.
4. *Twin pregnancy is as safe as a singleton* – there are risks associated with all pregnancies, however, there is overwhelming evidence that multiple gestations are higher risk.

The authors’ concluded by quoting from the ASRM Practice Committee report that the optimal outcome of an IVF cycle is a healthy singleton and the majority view seems to be in agreement that one embryo-one baby at a time is preferable.

In order to start to answer the cumulative pregnancy per cycle question I subsequently authored a study in 2013 which reviewed the implication of the Quebec policy change to eSET and the pregnancy rates per started COS. (*Velez et al*, 2013). In this study, IVF using COS cycles from one clinic in 2009 before the funding was introduced, were compared to IVF using COS cycles from the first twelve months of funding. The first frozen embryo transfer (FET) cycle was included in the data set for the funded cycles in order to compare DET in the 2009 cycles with one plus one in the funded cycles. Four hundred and ninety-nine stimulated IVF cycles were started in 2009 compared to 815 for the first year after implementation of funding; a 64% increase in uptake. Patient ages did not differ (an important point to be discussed in more detail later) (35.2 versus 35.4 years). The clinical pregnancy rate was significantly different when the fresh embryo transfer was compared (31.9% versus 23.3% p = 0.001) however when the first FET was included and cumulative pregnancy rate was used for the funded group there was no difference (31.9% versus 29.7%, p = 0.41). Furthermore, the proportion of cryopreserved embryos available per cycle increased between the groups (31.5% versus 57.2% in patients under 37, p < 0.001 and 7.2% versus 35.1% in patients over 37, p < 0.001) which offers even greater potential for additional pregnancies and increasing the final cumulative pregnancy rate in the funded cycles.

As previously mentioned, detractors of public funding and associated regulation on embryo number for transfer quote studies that demonstrate lower pregnancy rates when comparing SET with DET however these studies often include non-elective SET either exclusively or within the cohort of transfers. Studies which specifically analyse elective SET have shown similar results between eSET and DET outcomes (De Sutter *et al*, 2003). Clearly if eSET is the goal it is essential that the clinic has a strong embryo cryopreservation programme and the introduction of vitrification in human ART has permitted embryology laboratories to consistently produce survival rates in the high 90 percent with resultant clinical pregnancy rates that equal fresh transfer rates (Kuwayama, 2007). Vitrification was used exclusively for embryo cryopreservation for both the study groups in our analysis.

In Canada, professionals who work in the field of ART meet under the auspices of the Canadian Fertility and Andrology Society (CFAS) which, since 2000, has managed a national registry of ART
cycles performed. This registry is known as the Canadian ART Registry (CARTR) and since 2014 has been managed by Better Outcomes Registry Network (BORN). BORN is an Ontario government funded independent organisation charged with collating data on all birth outcomes for the province of Ontario. In their role of managing CARTR, BORN are responsible for collating data from across the country however clinics in the province of Ontario no longer need to supply birth outcomes from their IVF cycles since this information is collected directly by BORN from the hospitals. Clinics in the rest of Canada are still required to provide complete cycle information until birth since BORN can only receive patient identifiable data from within their province. Participation in the CARTR is not mandatory but all IVF clinics provide data and the database management is maintained by fees paid from the ART clinics. The CARTR data is available on request to researchers. The data necessary to carry out the studies comparing Quebec IVF before and after funding was obtained from BORN on application and having demonstrated the validity of the research questions being posed.

The other important element to consider with the government funding other than achieving a significant reduction in multiple pregnancy rate was to analyse the economic impact of the IVF coverage which I authored in 2014 (Velez et al., 2014). This study analysed the first complete calendar year of the programme, 2011, and compared the data with those cycles performed in the province during 2009 when patients had to self-fund their cycles and no legal limitations on treatment were applied.

In the economic analysis a model was developed based on the 1875 cycles from 2009 and 5489 cycles from 2011 as well as projecting live births from cryopreserved embryos based on 177 FET cycles in 2009 and 670 FET cycles in 2011. The financial comparison was made in consideration of the tax rebate given from 2009 as compared to the direct costs incurred by the government in 2011 with funding. In addition, costs associated with labour and delivery, preterm birth and neonatal care, as well as first year costs related to the child. Unsurprisingly the number of performed cycles increased by close to 200%, the age group representation of patients also changed with a reduction in patients under 35 years old and increase in those over 40 years old (this was only a transient effect as will be discussed later). As already demonstrated the multiple pregnancy rate dropped significantly (29.4% versus 6.4% p < 0.001) as did the clinical pregnancy rate (39.9% versus 24.9% p < 0.001). The costs to the government relative to IVF funding compared to a tax-rebate system increased from CAD$7 million to CAD$26 million in the first year and the total cost including antenatal care, birth and complication costs relative to IVF increased from CAD$31 million to CAD$41 million. Per IVF cycle the costs increased from CAD$3,730 to CAD$4,759, however, when considering all downstream costs related to health these decreased from CAD$16,747 to CAD$8,960 and based on projected numbers of
children born from the 2011 cohort compared to the actual live births from IVF and FET cycles in 2009, the cost per baby to one year of age actually decreased under the funding programme from CAD$49,517 to CAD$43,362 per live birth. This modeling suggested that in addition to reducing the multiple pregnancy rate or indeed perhaps because of its reduction the cost per cycle to the government per baby was less with funding than under a system of patient self-funding with a tax rebate in place. Our published model analysis did not take into account health care costs after a year of life which could arguably be more in the case of high order multiple deliveries, and it did not acknowledge the fiscal benefits brought by the children when they start to contribute to the economy themselves. Furthermore, it is important to note that the dramatic increase in cycles and therefore babies born, as a result of funding, balances somewhat the reduction in multiple births using neonatal intensive care facilities.

As a result of this series of publications I was asked along with my colleagues to provide a chapter for a book: Screening the Single Euploid Embryo: Molecular Genetics in Reproductive Medicine. (Ed: E Scott Sills, 2015). This book includes chapters on genetic analysis of embryos, as well as ovarian stimulation, sperm analysis, embryo selection techniques, embryo transfer techniques and analysis of several mandated eSET programmes. In our chapter we argue that cumulative pregnancy rate should be used as the best measure of IVF success (Velez et al, 2015). In order to analyse this, the cycles using elective double embryo transfer (eDET) from 2009 were assessed against the eSET cycles from 2011 plus the FET cycles using embryos created from those 2011 IVF cycles. Once again data was obtained from CARTR BORN. The patient ages were similar in the two periods (32.54 versus 32.52 p = 0.92), and age distribution was similar in the two groups, as was prior pregnancies and previous IVF attempts. There were some differences in the reason for treatment between the two groups with some indication that the eSET group was disadvantaged: lower mean number of oocytes retrieved, less cleaved embryos available, and fewer cryopreserved embryos. There were 514 eDET cycles performed in the province in 2009 with a pregnancy rate of 47.1%, and a multiple pregnancy rate of 35.1%. In 2011, there were 1375 eSET cycles with a pregnancy rate of 33.9% leaving 909 women to have a FET cycle: of these women, 378 completed their FET cycle in 2011 and a further 21.2% achieved a pregnancy. The cumulative pregnancy rate of the eSET patients was therefore 39.7% at this point, however, 478 women were still waiting for their FET cycle; if we assume 90% survival rate of the cryopreserved embryos (which is conservative considering vitrification was used in all cases), then an additional 101 pregnancies should be expected from these cycles. Including these additional pregnancies, the cumulative pregnancy rate of the eSET cohort would be 47.1%. This is equal to the 2009 eDET pregnancy rate, thereby demonstrating the argument that one embryo at a time achieves
the same outcome as DET and the importance of using cumulative pregnancy rate to assess success of eSET programmes.

Previously a prospective randomised trial comparing live birth rates using eSET plus FET against eDET confirmed that the multiple pregnancy rate can be significantly reduced (33.1% versus 0.8% p<0.001) and that any reduction in the live birth rate is unlikely to exceed 11.6 percentage points (42.9% versus 38.8%, difference 4.1 percentage points 95% C.I. -3.4 to 11.6) (Thurin et al, 2004). Our data demonstrated higher clinical pregnancy rates but within the same range if we consider some clinical pregnancy loss to live birth.

In 2015, after five and a half years, the Quebec government terminated the funding for IVF, and with the exception of fertility preservation for oncological reasons, IVF became patient-funded again in the province of Quebec. In 2018 I analysed the data from all the IVF cycles performed during the course of the programme and compared it to the combined 2009 and 2016 data: all data was again obtained from CARTR BORN (Bissonnette et al, 2019 in press). By combining data from 2009 and 2016 as the control group data it ensures that changes and improvements in technology over the course of the study are reduced as variables in the results. Since there were legislative changes that affected maximum numbers of embryos that could be transferred over the time period it also allowed me to assess the direct impact of the funding on results. Since there is birth registry linked to the CARTR database for the births in Quebec, the ART clinics are responsible for following up and obtaining this information. However, it is not always possible to obtain this information and there were 1310 ongoing clinical pregnancies without live birth information for the 2010-2015 data. Using a 15% loss of pregnancy from 8 weeks, an additional 1113 live births were assumed in the calculations, with an additional 27 similar cases for 2009 and 124 cases in 2016.

The total number of cycles increased dramatically as would be expected, averaging over 10000 for the funded years (reaching a maximum of 11900 in 2013) as compared to 2500 in 2009. In 2016 there were a total of 5353 IVF cycles of which 56% were FET since embryos created under funding remained funded for the FET. Of interest and importance, the proportion of age groups of patients treated did not differ significantly over the two periods. The cancellation rate increased which was related to the design of the funding: an IVF cycle did not count for the patient until embryo transfer, therefore creating an environment which led to aggressive cycle cancellation (17% versus 34.4% p < 0.001). This increase was seen in every age group with the exception of the patients over 43 years of age; possibly due to the acceptation that in this age group a better future outcome may not be achievable and so embryo transfer was carried out even with suboptimal embryo parameters. The use of donor
eggs reduced during funding (4.5% versus 2.4% p<0.001), once again probably associated with funding design: the cost of donor eggs was not included in the programme and so unless a patient could find an altruistic donor herself, she needed to resort to purchasing eggs from USA based egg banks at great financial cost. The use of donor semen, which was covered, also decreased (4.5% versus 2.4%, p<0.001) most likely because all aspects of IVF were funded including surgical sperm retrieval and ICSI thereby eliminating a major reason for some patients to consider intrauterine insemination using donor semen when diagnosed with a severe male factor.

The live birth rate per fresh embryo transfer decreased with funding confirming what we had previously published (33.9% versus 23.7% p < 0.001) however with this data set we did not calculate the cumulative or potential cumulative live birth rate. The live birth rate following treatment using MNCIVF also decreased (35.8% versus 25.2%, p=0.03) as did the live birth rate in FET (25.5% versus 21.9%, p<0.001). The use of eSET, which was a primary objective of the funding, increased in both the IVF with COS and the FET cycles (IVF 9.2% versus 64.3% p < 0.001, FET 10.0% versus 73.5% p < 0.001) when comparing funding with 2009 data and unsurprisingly remained high in 2016 (IVF with COS 71.5%, FET 86.7%) since legislation brought in at the end of funding actually tightened the legislation related to the number of embryos at transfer. As a result, the multiple pregnancy rate dropped from 25.6% to range between 3.3% and 7% during funding and remained under 5% in 2016.

Overall as a result of funding for 64 months there were 9232 live birth events (some of which were still multiple pregnancies therefore more than 9232 babies were delivered from this programme.

The major reason cited by the government for the closure of the programme was fiscal. It is difficult to obtain precise numbers concerning the actual costs however CAN$70 million to CAN$80 million per year were widely presented in the media. In our previous financial analysis of the programme in 2014, we did not consider the contribution element of the children born from such an ART funding programme. If one considers that the average tax contribution lifetime by an individual in the province of Quebec is approximately CAN$330 thousand (StatsCan), then the more than 9232 babies will provide over CAN$3 billion during the course of their lives. The 64 months of funding cost the government CAN$400 million which translates into an 8-fold return in their investment.

Clearly lessons can be learnt from the design of the funding programme which as mentioned encouraged cycle cancellation, for which the clinics were still paid and the patients did not lose their cycle. Providing a fixed number of cycles where the cycle counts once stimulation or at least egg collection is performed would provide for greater fiscal control while still giving access to treatment to all patients regardless of their socio-economic situation.
Chapter 5 – Conclusions

The publications presented here demonstrate some different approaches to achieving the objective of single embryo transfer and to offering treatment techniques that maximise the potential to offer ART care to as wide a population of patients as possible. Worldwide, the multiple pregnancy rate remains one of the major negative side-effects of IVF however, many countries have managed to control this either with legislation or simply a culturally driven agreement amongst ART practitioners that eSET is the best embryo transfer policy.

Clearly there have been a great number of developments that allow for eSET which have not been touched on in these publications. One of the most important amongst these is the significant improvements in IVF culture media permitting the possibility of prolonged culture and the transfer of a blastocyst rather than early cleavage stage embryos. Due to the natural selection process, being able to transfer a blastocyst increases the implantation rate per transfer thereby balancing to some degree the benefits of transferring more than one early cleavage stage embryo.

Research into improving embryo selection such as with the use of time lapse technology and pre-implantation genetic testing for aneuploidy (PGT-A), as well as other non-invasive assessments of culture media interaction and metabolomics have also allowed for greater understanding of the embryo and may improve selection parameters.

Furthermore general improvements in the understanding of the construction of an IVF laboratory including the air quality and materials used, developments on the pharmacological side of ART, previously mentioned developments in culture media complexity and increased knowledge about important events in embryo development using morphological indicators have led to increased outcomes from IVF which itself permits the option for eSET whilst maintaining high clinical pregnancy and live birth rates.

In terms of the impact that these publications have made; the number of citations for each publication is indicated on the cover page for each article at the end of this study. My publications and the ongoing use of MNCIVF at our clinic persuaded the government of Quebec to specifically include MNCIVF within the funding for IVF and based on our published data, permitting six cycles of MNCIVF as compared to three cycles of IVF with COS. This is the first government funding legislation to the best of my knowledge that specified MNCIVF within its terms. My publications on MNCIVF have been referenced in books such as the Development of In Vitro Maturation for Human Oocytes: Natural and Mild approaches to Clinical Infertility Treatment (2017, Springer International Publishing) and

The publications that I authored on *in vitro* maturation have been cited in multiple peer-reviewed articles and also referenced in books (*Infertility in Practice*, Informa Healthcare 2008) and in a consensus analysis of the PCOS condition by two of the major ART societies: *The ESHRE/ASRM consensus on polycystic ovary syndrome (PCOS) – an extended critical analysis* (Geisthovel *et al.*, 2007).

My publications related to public funding of IVF within the province of Quebec, Canada have been referenced for policy proposal documents both in the U.K. and the U.S.A.: *Elective single embryo transfer: an update to UK Best Practice Guidelines*, (Harbottle *et al.*, 2015, Human Fertility) and *Preterm births, multiples, and fertility treatment: recommendations for changes to policy and clinical practices* (Johnston *et al.*, 2014, Fertility and Sterility).

Further research is required to increase knowledge especially regarding improved embryo selection as well as investigating endometrial receptivity and thereby increasing implantation potential. These developments would permit eSET to become the standard for ART without risk of reducing live birth rates especially in combination with cryopreservation which already appears to have reached extremely high efficacy.
References

References in **bold** contribute to the body of published work submitted in evidence for the award of PhD by Publication.


Kadoch IJ, Phillips SJ, Bissonnette F. Modified natural cycle in vitro fertilization should be considered as the first approach in young poor responders. Fertil. Steril. 2011. 96(5):1066-1068


Shahin A. The problem of IVF cost in developing countries: has natural cycle IVF a place? RBMOnline. 2007 15(1):51-56


Velez MP, Kadoch IJ, Phillips SJ, Bissonnette F. Rapid policy change to single-embryo transfer while maintaining pregnancy rates per initiated cycle. RBMOnline. 2013. 26:506-511


Additional Published Works by the Author

Scientific publications


Dean NL, Phillips SJ, Buckett WM, Biljan MM, Tan SL. Impact of reducing the number of embryos transferred from three to two in women under the age of 35 who produced three or more high quality embryos. Fertil. Steril. 2000. 74:820-823


Published Abstracts


Bissonnette F, Phillips S, Holzer H, Mahutte N, St-Michel P, Gunby J, Kadoch IJ. Funding is the most powerful tool against multiple pregnancies in ART. Hum. Reprod. 2011. 26 suppl1: i113-i114


Delrieu D, Himaya E, Saumet J, Dzineku F, Phillips S, Velez MP, Kadoch IJ. Monozygotic multiple pregnancies following assisted reproductive technology: is it the time to start an international registry? Hum. Reprod. 2012. 27 ii95-ii98

El Hachem H, Lefebvre J, Antaki R, Sylvestre C, Bissonnette F, Phillips S, Dean N, Benoit J, Lapensee L. Controlled ovarian stimulation (COS) with 450 IU/day vs 600IU/day in poor responders: A secondary analysis according to the Bologna criteria. Fertil. Steril. 2015. 104(3) e52-e53


Khudhari A, Hemmings R, Phillips S, Badeghiesh AM, Jamal W. How does ART singletons differ from naturally conceived (nc) singletons; comparison of perinatal data of 872 ART to 19317 (nc) singleton babies. Fertil. Steril. 2016. 106(3) e176-e177

Kutbi B, Phillips S, Lapensee L. What is the best methodology to train and evaluate the training of reproductive endocrinology and infertility fellows for embryo transfers? Fertil. Steril. 2017. 108(3) e235


Lesaint C, Vingataramin L, Alix S, Phillips S, Zini A, Kadoch IJ. Correlation between two sperm DNA fragmentation tests (TUNEL and SCSA) and evaluation of TUNEL assay inter-labs variability. Fertil. Steril. 2016. 106(3) e297


Phillips S, Courchesne A, Haddak K, Bissonnette F, Kadoch IJ, Dean N. Prospective observational comparison of traditional slow freezing with a closed vitrification system. RBMOnline. 2010. 20 S51-S52


Appendix 1


**Contribution by SJ Phillips**

Concept  
Data collection  
Data analysis  
Manuscript writing and editing

**Citation Metrics**

Google Scholar: 29  
Scopus: 23  
Web of Science: 21
Article

Controlled natural cycle IVF: experience in a world of stimulation

Simon Phillips graduated from the University of Plymouth in England. He started his career as a clinical embryologist working in London at the Hallam Medical Centre. He later moved to Canada to work at McGill University and further develop his knowledge and techniques in the field of assisted reproduction. Since 2003 he has been the Laboratory Director at the OVO Fertility Clinic in Montreal, which he helped to start. His interests lie in improving many aspects of clinical embryology especially embryo selection and quality. He is a member of the ASRM, ACE, CFAS and CFAS ART SIG executive.

Dr Simon Phillips

Simon J Phillips1,3, Isaac Jacques Kadoch1,2, Louise Lapensée1,2, Bernard Couturier1, Robert Hemmings1, François Bissonnette1,2

1OVO Fertility Clinic, 8000 boulevard Décarie, Montréal, Québec, Canada H4P 2S4; 2University of Montreal, Department of Obstetrics and Gynecology, Saint Luc Hospital (CHUM), 1058 rue Saint-Denis, Montréal, Québec, Canada H2X 3J4

Correspondence: e-mail: s.phillips@cliniqueovo.com

Abstract

A total of 134 controlled natural IVF (nIVF) cycles were reviewed retrospectively and compared with 370 stimulated IVF (sIVF) cycles. The clinical pregnancy rate per embryo transfer following nIVF was 27% and 47% in sIVF cycles for patients aged less than 35. However, natural cycle patients could attempt consecutive cycles with much less impact on their lives, both medically and financially. In patients under 35 years of age, the choice of controlled nIVF reduces the cost and risk to the patient, permitting her to have multiple, consecutive attempts, and cumulatively offers a clinical pregnancy rate which approaches that of sIVF. The multiple pregnancy rate in nIVF is significantly reduced compared with sIVF treatment cycles. In patients over 35 years of age the benefits of nIVF were much less evident (clinical pregnancy rate: 8% per embryo transfer) and the opportunity to transfer multiple embryos in these patients seems to be advantageous.

Keywords: clinical pregnancy rate, IVF, multiple pregnancies, natural cycle

Introduction

Over recent years, in-vitro fertilization has worked on the principle of stimulation of the ovaries and the production of multiple follicles in order to obtain many oocytes and, hopefully, several embryos. From these embryos, a choice is made as to which ones offer the best developmental potential, and these are then transferred to the patient’s uterus. However, the first IVF baby was conceived following a natural cycle (Steptoe and Edwards, 1978) and indeed natural cycle IVF (nIVF) was the method of choice for the first few years of IVF treatment.

The use of ovarian stimulation allowed an increase in the number of recruited oocytes and an increase in the pregnancy rates associated with IVF (Fishel et al., 1985). It also permits excess embryos to be cryopreserved in the event that the IVF attempt fails to result in a pregnancy or, indeed, to allow the patient to return for additional sibling attempts in the future without a need for further ovarian stimulation. However, due to the additional costs, risks and complexity of ovarian stimulation, there is pressure on both the patient and the care giver to increase the chance of the patient becoming pregnant and therefore it has become standard to transfer more than one embryo. A side effect of this is a dramatic increase in the rate of multiple pregnancies, which has been observed. Although some countries have acted by law to restrict the number of embryos that are permitted to be transferred, such as in the UK with the Human Fertilization and Embryology Authority, many countries leave the decision in the hands of the assisted reproduction professionals and their patients.

Although various different methods have been suggested as to how to choose the best embryos for embryo transfer, such as embryo morphology, nutrient uptake or pronucleus appearance,
there is still no absolute method for determining which embryo has the best chance of developing into a successful pregnancy.

In a natural cycle, several follicles are recruited initially but it is only one that attains dominance and goes on to ovulate. Being able to control ovulation was one of the reasons why natural cycles were replaced with ovarian stimulation cycles, because the first oocyte retrievals had to be performed according to the natural LH surge and resulted in collections at any time of the day or night in order to collect the oocyte before ovulation.

Developments of gonadotrophin-releasing hormone (GnRH) antagonists allowed once again the possibility of using a natural cycle, by preventing the occurrence of a premature LH surge and thereby allowing better control (Rongières-Bertrand et al., 1999). More recently, Trokoudes et al. (2005) published a series of controlled nIVF cycles (CONCIVF) in which he showed that CONCIVF could be a useful tool in the management of some patients presenting for fertility treatment.

Materials and methods

Patients under 39 years of age attending the OVO Fertility Clinic from January 2004 were offered the possibility of having IVF via a controlled natural cycle or stimulated IVF. Those choosing to accept controlled nIVF had a transvaginal ultrasound on day 6 and then serial ultrasounds to measure the leading follicle. It should be noted that patients who had their cycles cancelled before oocyte collection due to premature ovulation or abnormal follicular growth were excluded from the results.

Ultrasound scans were performed using a multifrequency transvaginal probe (Voluson 730 Expert, GE Medical Systems, Saint-Laurent, Quebec, Canada). Assessments were made by measuring the follicle in two perpendicular planes and taking the average diameter. Once the follicle reached 14 mm, GnRH antagonist (Ganirelix, Orgalutran, Organon Pharmaceuticals, Scarborough, Ontario, Canada) was administered (0.25 mg) as a s.c. injection daily until the day of human chorionic gonadotrophin (HCG) administration. Also patients received 150 IU of human menopausal gonadotrophin (HMG) (Repronex, Ferring Canada, North York, Ontario, Canada) daily. The use of HMG was due to the study of Kettel et al. (1991) to prevent an oestradiol drop in response to the GnRH antagonist. No stimulatory effect of HMG was seen: indeed no follicles reached an 18 mm average of two perpendicular planes, 10,000 IU of HCG (Pregnyl; Organon Pharmaceuticals) was given and oocyte retrieval was scheduled for 36 h later.

Standard insemination or intracytoplasmic sperm injection (ICSI) was performed according to well-established sperm parameters. Fertilization was verified by the presence of two pronuclei and two polar bodies 18 h after insemination. All fertilized oocytes were transferred into cleavage media (Cook). Embryo transfer was performed under ultrasound guidance using a Wallace Sureview catheter (Smith Medical, UK) on day 2 after oocyte retrieval. Embryo transfer for stimulated IVF cycles was on day 2 or 3 after oocyte retrieval, depending on the numbers of embryos available.

Patients received two injections of HCG (2500 IU), on day 2 and day 4 after collection, and took progesterone intravaginally 600 mg daily (Prometrium, Schering, Pointe-Claire, Quebec, Canada), starting 2 days after oocyte retrieval, and continued until menstruation or for at least the first 8 weeks of pregnancy if the patient became pregnant.

Pregnancy was verified by serum HCG 15 days after oocyte collection and confirmed by the presence of an intrauterine fetal heartbeat by ultrasound at 6 weeks.

Results

Between January 2004 and October 2006, patients attending the clinic for IVF treatment were offered the possibility of sIVF or controlled nIVF. Patients over the age of 35 were counselled and advised to choose IVF in preference, although patients who insisted on trying nIVF were accepted. The data for the results with nIVF are reported in Table 1.

In summary, 134 cycles were performed for patients under 35 years of age, with 75 resulting in an embryo transfer. The clinical pregnancy rate per embryo transfer was 27%. As a comparison, during the same time period, 370 cycles of sIVF were performed for patients under the age of 35 with 355 resulting in an embryo transfer. The clinical pregnancy rate per embryo transfer was 47%. However, in the sIVF group the multiple pregnancy rate was 37% (Table 2).
For those patients over 35 who insisted on attempting nIVF, there were 108 cycles resulting in 52 embryo transfers. The clinical pregnancy rate per transfer was 8%. In sIVF for the same age of patient, there were 240 cycles with 220 embryo transfers and the clinical pregnancy rate per embryo transfer was 37%. The multiple pregnancy rate in this group was 36%.

The cancellation rate or failure to reach embryo transfer was considerably higher in the controlled nIVF groups than in the sIVF groups. For the two treatment groups under 35, 59% of patients under 30 and 54% of patients between 31–35 reached embryo transfer as compared with 96% of patients in sIVF.

The 134 cycles of nIVF were performed for 70 patients. The clinical pregnancy rate per patient undergoing nIVF was 28.5% and 91% of the patients becoming pregnant did so on their first or second attempt, including cycles with no embryo transfer.

### Conclusions

Although controlled nIVF does not reach the levels of pregnancy rate that can be obtained by transferring multiple embryos following ovarian stimulation, the authors believe that it can be a useful tool in the treatment of some couples presenting for infertility. The benefits of controlled nIVF are obvious, in that the increasing problem of multiple pregnancy is removed, the patients do not need to inject themselves with expensive medications, and due to the reduced invasive nature of the procedure (no sedation or anaesthesia is used for the oocyte collections), patients can, if they choose, have a cycle each consecutive month. If it is also considered that, as with intrauterine insemination, patients can have three cycles of nIVF consecutively with minimal impact on their life schedules, then the cumulative pregnancy rate will approach that of sIVF cycles. Since it was found that the majority of pregnancies in nIVF occur on the first or second attempt (91%), including cycles with no embryo transfer, most patients are counselled to use sIVF after three unsuccessful attempts with nIVF.

Also it appears that the benefits of transferring more than one embryo are increased in patients over the age of 35, since the pregnancy rate from nIVF was low (8%). In the sIVF group, patients over the age of 35 had a pregnancy rate that was comparable with the younger sIVF groups (37% for patients over 35 versus 53% for patients less than 30, and 44% for patients aged 31–35). It has been suggested that vaginal oestradiol supplementation may improve implantation rate (Wright et al., 2006); however, no benefit in controlled nIVF was seen in this clinic (unpublished data).

While laboratory researchers are trying to find the best method

### Table 1. Results of controlled natural cycle IVF (nIVF).

<table>
<thead>
<tr>
<th>Patient age (years)</th>
<th>&lt;31</th>
<th>31–34</th>
<th>35–38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>44</td>
<td>90</td>
<td>108</td>
</tr>
<tr>
<td>Number of embryo transfers</td>
<td>26</td>
<td>49</td>
<td>52</td>
</tr>
<tr>
<td>Cycles leading to embryo transfer (%)</td>
<td>59</td>
<td>54</td>
<td>48</td>
</tr>
<tr>
<td>Cycles with no oocytes retrieved (%)</td>
<td>23</td>
<td>24</td>
<td>32</td>
</tr>
<tr>
<td>Cycles with no fertilization (%)</td>
<td>18</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Number of clinical pregnancies</td>
<td>7</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td>Clinical pregnancy rate per embryo transfer (%)</td>
<td>27</td>
<td>27</td>
<td>8</td>
</tr>
</tbody>
</table>

### Table 2. Results of stimulated IVF (sIVF).

<table>
<thead>
<tr>
<th>Patient age (years)</th>
<th>&lt;31</th>
<th>31–34</th>
<th>35–38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>145</td>
<td>225</td>
<td>240</td>
</tr>
<tr>
<td>Number of embryo transfers</td>
<td>139</td>
<td>216</td>
<td>220</td>
</tr>
<tr>
<td>Cycles leading to embryo transfer (%)</td>
<td>96</td>
<td>96</td>
<td>92</td>
</tr>
<tr>
<td>Number of clinical pregnancies</td>
<td>74</td>
<td>94</td>
<td>81</td>
</tr>
<tr>
<td>Clinical pregnancy rate per embryo transfer (%)</td>
<td>53</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td>Multiple pregnancy rate (%)</td>
<td>38</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>Average number of embryos transferred</td>
<td>2.1</td>
<td>2.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>
to select an embryo or embryos with the best developmental potential for embryo transfer, perhaps the role of natural selection of follicles should be considered. Further research is necessary to verify whether the aneuploidy rate, or perhaps nutrient uptake or output, in natural cycle embryos is improved over embryos resulting from stimulation cycles.

Since there is only one follicle in nIVF, there is a greater risk of cycle cancellation than in stimulated cycles, even in younger patients. This is an area of controlled nIVF that needs more attention and research, since there is certainly less chance of an embryo transfer following the commencement of a controlled nIVF cycle than a sIVF cycle. From the data presented here, slightly over half the cycles in controlled nIVF result in an embryo transfer (59%, 54%) as compared with nearly all cycles in sIVF (96%). The use of non-steroidal anti-inflammatory drugs (NSAID) has been proposed to improve the recuperation of oocytes by reducing the spontaneous ovulation rate (Nargund et al., 2001), and this is an area currently under investigation in the clinic’s programme. In addition, methods to improve fertilization rate with ICSI may provide helpful in ensuring that more patients complete their cycles (Wang et al., 2001; Hazout et al., 2006).

However, the choice to use nIVF can be incorporated into the financial aspect of the programme so that the impact on patients is reduced when an embryo transfer does not occur. For example, in this clinic, patients who do not obtain an oocyte at retrieval pay a much-reduced price simply to cover the costs of the retrieval only, and if the oocyte fails to fertilize, a significant rebate is also given on the cycle price. Patients who have their cycles cancelled before oocyte collection due to premature ovulation or abnormal follicular growth do not pay for their cycle at all, and so they have been excluded from these data. Therefore, only patients who have an embryo transferred pay the total cycle costs, which are still considerably less than a stimulated cycle. It is for this reason that clinical pregnancy rate per embryo transfer is reported, rather than the more traditional clinical pregnancy rate per cycle started. While the authors realise that describing clinical pregnancy rates per embryo transfer may be construed as misleading, they feel that controlled nIVF is sufficiently different to sIVF in terms of impact on the patient’s life to warrant this. Perhaps in a situation where there is no or little financial implication on patients for fertility treatment, this argument is reduced; however, certainly in North America where, unfortunately, the financial burden of assisted reproduction falls on the patients and not governments or insurance companies, the authors feel that it is reasonable to present clinical pregnancy rates per embryo transfer as long as the patients are aware that the chance of failing to reach embryo transfer in a controlled nIVF is higher. During failed cycles, patients will, of course, take very little, if any medication, and undergo two or three vaginal ultrasounds at most, and the costs will be much reduced. Trokoudes et al. (2005) also used clinical pregnancy rates per embryo transfer in their controlled nIVF paper, suggesting that perhaps nIVF should be analysed in a slightly different way to sIVF. Patients initiating controlled nIVF at the centre are counselled about the risk of failing to reach embryo transfer, which may be up to 50% of those starting a cycle, although this rate is expected to improve with research involving NSAID, as mentioned previously, and with potential improvements to the protocol in the future.

The authors believe that, using the method that is described here, that a successful alternative to sIVF can be offered to patients and, in doing so, the various burdens that sIVF places on the patient and health care systems be considerably reduced.

Acknowledgements

The authors would like to thank all members of the OVO Fertility Clinic team for their support.

References


Received 2 November 2006; refereed 8 December 2006; accepted 12 January 2007.
Appendix 2


Contribution by SJ Phillips
- Concept
- Manuscript writing and editing

Citation Metrics
- Google Scholar: 4
- Scopus: 3
- Web of Science: 3
Natural cycle IVF: a question of semantics?

Simon John Phillips1, Isaac Jacques Kadoch
OVO Fertility Clinic, Montréal, Québec, Canada
1Correspondence: e-mail: s.phillips@cliniqueovo.com

Abstract

Recently there has been much discussion and presentation on IVF protocols using less stimulation or indeed none at all. Our experience with controlled natural cycle IVF over the last few years has convinced us that this is a powerful tool for many patients in the treatment of infertility. The protocol we employ has raised some questions as to whether it is natural cycle or stimulated cycle. We have reported a large series of cycles and seen no stimulatory effects of the medications used to control the cycle, thereby confirming our position that controlled natural cycle IVF is a valid option as an assisted reproduction treatment.

Keywords: controlled natural cycle IVF, IVF, minimal stimulation

There is a returning interest to natural cycle IVF and minimal stimulation regimes as well as in-vitro maturation. They are all useful tools in the treatment of infertility and, as recently suggested in this journal, may be the future of assisted reproduction technologies (Edwards, 2007; Nargund and Frydman, 2007; Ubaldi et al., 2007).

We have been working with controlled natural cycle IVF for several years and, indeed, have published articles on the subject in this journal (Reproductive BioMedicine Online) (Kadoch et al., 2005, 2007a,b; Phillips et al., 2007). One issue that has repeatedly arisen when articles are submitted is the question of whether the protocol we employ is truly natural cycle IVF. The terminology of the protocols has become a question of debate.

As discovered by IVF pioneers, such as Professor Edwards, truly natural cycle IVF is not an efficient treatment protocol due to the need for 24-hour oocyte retrieval and laboratory coverage, and the increased risks of premature ovulation. The advent of gonadotrophin-releasing hormone (GnRH) antagonists provided the opportunity to revisit the natural cycle in a more controlled manner; however, evidence of a negative effect on oestradiol concentrations once GnRH antagonists are administered throws up an additional complication. The use of small doses of human menopausal gonadotrophin (HMG) to counteract these negative effects of GnRH antagonist allows for a truly ‘controlled’ natural cycle protocol (Kettel et al., 1991).

In the protocol that we have been employing, HMG is administered at 150 IU upon commencement of GnRH antagonist. The patients, on average, only take HMG for 2 or 3 days and, in nearly 400 cycles, we have never seen a stimulatory effect from the gonadotrophins.

The new protocols of minimal stimulation and ‘friendly IVF’ that have been proposed recently are also useful and interesting protocols. However, these protocols are designed to promote multi-follicular development and co-dominance, exactly the opposite of the intention of our controlled natural cycle protocol (Olivennes and Frydman, 1998; Macklon and Fauser, 2003). We believe that the ‘natural’ selection of a follicle, and thus an oocyte, is an important tool at a time when research to select the ‘best’ embryo for transfer is ongoing.

Our studies with controlled natural cycle IVF have shown that it is a powerful protocol for use in patients less than 35 years of age. However, the minimal stimulation protocols may well be beneficial to older patients, who appear to need more than one embryo or, at least, perhaps a choice at embryo transfer.

We are confident in the terminology of our protocol as ‘controlled natural cycle IVF’ rather than as a protocol of ‘minimal stimulation’.

Nevertheless, regardless of the precise terminology used, the overall philosophy of moving towards less stimulation and less embryos transferred must be commended and encouraged.

References


Kadoch IJ, Al-Khaduri M, Phillips SJ et al. 2007b Spontaneous ovulation rate before oocyte retrieval in controlled natural cycle in-vitro fertilization (nIVF) with and without indomethacin. Reproductive BioMedicine Online. Accepted for publication.


Kettel LM, Roseff SJ, Chiu TC et al. 1991 Follicular arrest during the midfollicular phase of the menstrual cycle; a gonadotropin-releasing hormone antagonist imposed follicular–follicular


Received 29 May 2007; refereed 11 June 2007; accepted 15 June 2007.
Appendix 3


**Contribution by SJ Phillips**
- Data collection
- Data analysis
- Manuscript writing and editing

**Citation Metrics**
- Google Scholar: 32
- Scopus: 21
- Web of Science: 13
Spontaneous ovulation rate before oocyte retrieval in modified natural cycle IVF with and without indomethacin

Dr Isaac Jacques Kadoch

Isaac Jacques Kadoch1,2,4, Maha Al-Khaduri1,2,3, Simon J Phillips1, Louise Lapensée1,2, Bernard Couturier1, Robert Hemmings1, François Bissonnette1,2
1OVO Fertility Clinic, 8000 Boulevard Décarie, Montréal, Québec, Canada H4P 2S4; 2University of Montreal, Department of Obstetrics and Gynecology, Saint Luc Hospital (CHUM), 1058 rue Saint-Denis, Montréal, Québec, Canada H2X 3J4; 3Sultan Qaboos University, Department of Obstetrics and Gynecology, Sultan Qaboos University Hospital (SQUH), Al-Khod, Sultanate of Oman
4Correspondence: e-mail: ij.kadoch@umontreal.ca

Abstract

The objective of this retrospective analysis was to evaluate the number of spontaneous ovulations occurring before oocyte retrieval in natural cycle IVF (nIVF) with and without the use of indomethacin. A total of 121 patients who underwent modified nIVF cycle between December 2003 and July 2006 were included in the study; 171 cycles without indomethacin and 84 cycles with indomethacin, started when the leading follicle reached 14 mm in size, were compared. The number of cycles with ovulation before oocyte retrieval and the number of cycles with no oocytes at retrieval were assessed with and without indomethacin. In addition, the pregnancy rates in the two groups of patients were analysed. There were 28 cycles (16%) in which ovulation occurred before oocyte retrieval in the group where no indomethacin was used and five cycles (6%) in which ovulation occurred before retrieval in the group where indomethacin was used. There was a statistically significant association between premature ovulation and indomethacin, with an odds ratio of 3.8 (95% confidence interval, 1.2–12.3).

The oocyte retrieval per started cycle was 64% without indomethacin and 76% with indomethacin (P < 0.04). The clinical pregnancy rate per embryo transfer was 14% without indomethacin and 21% with indomethacin (not significant).

Keywords: human oocyte, indomethacin, modified natural cycle IVF, oocyte retrieval, premature ovulation

Introduction

In recent years, natural cycle IVF (nIVF) has been largely replaced by conventional stimulated IVF. With ovarian stimulation, multiple follicles are recruited and many oocytes can be retrieved at the time of collection. This technique is advantageous, since multiple embryos are produced, which provides the opportunity to try to choose those that will give the patient the best chance of successful pregnancy. Conventional stimulated IVF has increased the pregnancy rates achieved, but since more than one embryo is usually transferred at a time, it has also increased the rate of multiple pregnancies. This is now considered a major complication, due to the increased morbidity and mortality associated with neonatal prematurity.

The search for ways to reduce multiple pregnancies resulting from assisted reproduction has renewed interest in nIVF. In nIVF, the selection of the dominant follicle is natural and devoid of any stimulation, so only one oocyte is retrieved and fertilized to produce a single embryo. This has several advantages: close to zero multiple pregnancy rate; avoids the risk of ovarian hyperstimulation syndrome; is less time consuming; physically and emotionally less demanding for patients; costs less than conventional stimulated IVF; and cycles can be done in consecutive months without a rest period as the ovaries are not stimulated.
The efficacy of nIVF, however, is hampered by high cancellation rates of up to 30% because of premature LH rise and premature ovulation before retrieval, which is not seen in conventional stimulated IVF. There have been several attempts to prevent the premature ovulation associated with nIVF. Claman et al. (1993) administered human chorionic gonadotrophin (HCG) in 75 nIVF cycles in an effort to preempt the LH surge. This was unsuccessful, as the cycle cancellation rate was 47% due to ovulation. Another attempt to prevent the LH surge was made using a gonadotrophin-releasing hormone (GnRH) antagonist (cetrorelix) (Rongières-Bertrand et al., 1999). This was done in a pilot study of 44 cycles with cetrorelix administered in the late follicular phase. The cycle cancellation rates due to premature ovulation were much lower than previously reported, at just 9%. This was evidence that GnRH antagonists may allow better control of the natural cycle, but did not eliminate the problem of premature ovulation.

There is evidence in the literature to support the use of indomethacin to reduce the incidence of spontaneous ovulation in nIVF. Nargund and Wei (1996) reported a case in which ovulation was successfully delayed for 1 week with the use of indomethacin at a dose of 50 mg three times a day for a total of 7 days. These workers later showed a lower spontaneous ovulation rate in a prospective trial comprising 42 nIVF cycles on indomethacin (50 mg three times daily for 3 days), compared with 139 cycles in which no indomethacin was administered (Nargund et al., 2001). Although the difference was not significant between the two groups, the patients who took indomethacin were able to delay oocyte retrieval for up to 72 h.

Indomethacin, a non-steroidal anti-inflammatory drug (NSAID), has well known anti-prostaglandin effects. NSAID act mainly through the inhibition of cyclooxygenase, the enzyme that catalyses the synthesis of prostaglandins, which are essential mediators of ovulation, implantation and placentation of the conceptus. Prostaglandins induce the mobilization of granulosa and theca interna cells within the ovaries, and the cyclooxygenase-2-dependent prostaglandins probably lead to the generation of proteolytic enzymes that rupture the follicles (Athanasiou et al., 1996). Use of NSAID has been associated with the luteinized unruptured follicle syndrome (Mendonca et al., 2000).

Athanasiou et al., (1996) have shown that indomethacin administered at the time of a positive urinary LH can delay follicular rupture, with an associated reduction in intrafollicular blood flow but with no apparent effects on hormonal or menstrual status. The mechanism of action of indomethacin, therefore, is probably inhibition of the ‘inflammation’ associated with follicular rupture. Unlike GnRH antagonists it does not inhibit the LH surge. Furthermore, it is associated with much lower costs.

In this study, it is proposed that use of indomethacin in nIVF would reduce premature ovulation, therefore reducing cycle cancellations and improving the efficacy of nIVF.

Materials and methods

Patients

Between December 2003 and July 2006, 121 patients (255 cycles) underwent nIVF at OVO Fertility Clinic. The database and charts were reviewed for details of the cycles. There were 171 cycles without indomethacin and 84 cycles in which indomethacin was used. All patients had normal ovulatory cycles (27–31 days). Inclusion criteria included male factor infertility, tubal obstruction, implantation failure in conventional IVF, young women with reduced follicular ovarian reserve and history of previous hormone-dependant cancer.

Protocol

All patients were observed using serial transvaginal sonography starting from cycle day 6. Assessment of the leading follicle was made by taking the average of two perpendicular measurements. When the follicle reached 14 mm in size, all patients received 0.25 mg subcutaneously of a GnRH antagonist (Ganirelix, Orgalutran; Organon, Ontario, Canada) daily until the day of HCG injection. Patients who were in the indomethacin group received 50 mg of indomethacin (Indocid; Merck Frost, Quebec, Canada) three times a day until the day before oocyte retrieval. All patients also received 150 IU of human menopausal gonadotrophin (HMG; Repronex, Ferring Canada, Ontario, Canada) daily to sustain oestriadiol concentrations. No stimulatory effect was seen from the administration of gonadotrophins, and their use was simply to prevent an oestradiol drop once GnRH antagonist was administered (Kettel et al., 1991). Once the follicular size reached 17 mm or greater, 5000 IU of HCG (Pregnyl; Organon) was administered subcutaneously. Oocyte retrieval took place 34 h later. On the day of retrieval, endovaginal sonography was performed prior to collection to confirm the presence of a follicle. Absence of a follicle was considered to be due to premature ovulation. Oocyte retrieval was performed under transvaginal ultrasound guidance using a 17G double lumen needle (Cook, Canada) and the follicle flushed as necessary (follicle flushing buffer, Cook). The procedure was performed without sedation or local anaesthesia. The oocytes were collected into fertilization media (Cook) at 37°C, under 6% CO₂. The partner’s semen was processed using a gradient system (Puresperm; Nidacon Ltd, Sweden). If necessary, spermatozoa were retrieved by percutaneous sperm aspiration (PESA), usually performed before the nIVF cycle and the spermatozoa cryopreserved (sperm maintenance media, Irvine Scientific, USA). Standard insemination or intracytoplasmic sperm injection (ICSI) was performed. Fertilization was verified by the presence of two pronuclei and two polar bodies 18 h post-insemination or ICSI. All fertilized oocytes were transferred into cleavage media (Cook). Embryo transfer was carried out under ultrasound guidance using a Wallace Sureview catheter (Smith Medical, UK) on day 2 post-oocyte retrieval. Patients received two subcutaneous injections of HCG, 2500 IU each, on days 2 and 4 post-collection. Patients were also given intravaginal progesterone (Prometrium; Schering, Quebec, Canada) 600 mg daily, starting 2 days post-retrieval and continued until the pregnancy test. If the patient became pregnant, she was continued on Prometrium until the eighth week of pregnancy. Pregnancy was verified by serum HCG measurement 15 days post-oocyte collection and confirmed by the presence of a fetal heart at 6 weeks of gestation via transvaginal sonography.

Statistical analysis

Statistical analysis was performed using the SAS system. Parameters at baseline between the two groups were compared using Student’s t-test. The analysis of categorical data was by the chi-squared test and Fisher’s exact test. A multivariate
logistic regression was used for outcome measures. The level of significance was set at $P < 0.05$ and two tailed tests were used.

**Results**

There were 84 cycles with indomethacin and 171 without indomethacin. The baseline characteristics between the two groups were similar for age of patients, attempt number, cause of infertility, reduced ovarian reserve, previous implantation failure, ICSI, IVF and sperm source (Table 1).

The primary outcome was the premature ovulation rate, which in the indomethacin group was 6% (5/84) as compared with 16% (28/171) in the non-indomethacin group. There was a statistically significant association between indomethacin and ovulation ($P = 0.02$, OR = 3.8, 95% CI = 1.2–12.3), so that the odds of ovulation with no indomethacin were almost 4 times the odds of ovulation with indomethacin.

The oocyte retrieval rate per cycle was 76% (64/84) in the indomethacin group and 64% (109/171) in the no indomethacin group ($P = 0.04$), which was statistically significant. This is probably due to reduced premature ovulation as a result of indomethacin. The oocyte retrieval rate per retrieval procedure, however, was not altered significantly: 81% (64/79) and 76% (109/143) in the indomethacin and no indomethacin groups respectively. The adjusted odds ratio for no oocytes at retrieval per collection procedure with indomethacin versus no indomethacin was 0.9 (CI = 0.4–1.7), which was not statistically significant.

The embryo transfer rate per collection was not significantly altered by the use of indomethacin, being 52% (41/79) in the indomethacin group and 55% (78/143) in the no indomethacin group. The clinical pregnancy rate per cycle, embryo transfer and oocyte retrieval procedure were increased in the indomethacin group, but the difference was not statistically significant between the two groups (Table 2). The clinical pregnancy rate per embryo transfer is the more important parameter to look at in nIVF, due to cycle cancellation secondary to premature ovulation. There was a lower pregnancy rate per embryo transfer in the group with no indomethacin as compared with the group on indomethacin. This difference was not statistically significant, however.

The pregnancy rate per embryo transfer was further analysed by age, as the cumulative pregnancy rate for all ages seemed to be low. The clinical pregnancy rate per embryo transfer for women 35 years of age or less was 31% (8/26), and 14% (7/50) for those over 35 years old. For women over 35 years old, the clinical pregnancy rate was 6% (1/16) on indomethacin and 13% (4/30) without indomethacin. There was a non-significant trend towards an increase in the pregnancy rate per embryo transfer in these younger women as compared with women over 35 years of age. The adjusted odds ratio for the clinical pregnancy rate per embryo transfer was 0.6 (95% CI = 0.1–2.5) which was not statistically significant. The clinical pregnancy rate per embryo transfer was 52% (41/79) in the indomethacin group and 55% (78/143) in the no indomethacin group.

There was no significant association between indomethacin and implantation. The implantation rate per embryo transfer with

| Table 1. Comparison of patient characteristics in the indomethacin versus no indomethacin groups. |
|-----------------------------------------------|-----------------------------------------------|
| **Patient characteristic** | **Cycles with indomethacin** (n = 84) | **Cycles without indomethacin** (n = 171) |
| Mean age ± SD (years) | 34 ± 3.2 | 34 ± 3.5 |
| Mean no. of attempts ± SD | 1.8 ± 1.3 | 2.0 ± 1.0 |
| Cause of infertility n (%) | | |
| Unexplained | 21 (25) | 38 (22) |
| Male factor | 43 (51) | 105 (61) |
| Endometriosis | 9 (11) | 10 (6) |
| Tubal factor | 11 (13) | 18 (11) |
| Reduced ovarian reserve | 14 (17) | 31 (18) |
| Previous implantation failure (conventional IVF) | 2 (2) | 0 (0) |
| No. receiving ICSI | 48 (57) | 110 (64) |
| No. receiving IVF | 36 (43) | 61 (36) |
| Assisted hatching | | |
| Yes | 17 (20) | 71 (42) |
| No | 67 (80) | 100 (58) |
| Sperm source | | |
| Ejaculate | 53 (63) | 103 (60) |
| PESA | 11 (13) | 11 (6) |
| TESA | 3 (3) | 3 (2) |

ICSI = intracytoplasmic sperm injection; PESA = percutaneous epididymal sperm aspiration; TESA = testicular sperm biopsy.
indomethacin was 13% (11/85) and without indomethacin was 8% (14/184).

**Discussion**

One of the main disadvantages of nIVF, causing it to fall out of favour, is the associated high rate of premature ovulation and associated cycle cancellation, which results in a range of consequences, including time wastage and costs. The results obtained from this study, together with evidence from previous studies, suggests that the use of indomethacin may play an important role in overcoming one of the major obstacles in nIVF. In addition, the safety profile and the low cost of this medication make it attractive to use.

Results from this study show that the use of indomethacin in nIVF reduces premature ovulation from 16% (without indomethacin) to 6%. Patients who did not receive indomethacin had four times the odds of premature ovulation than those who had received indomethacin during the nIVF cycle. This led to a significant increase in oocyte retrieval rate per started cycle as a result of a reduction in cycle cancellation due to premature ovulation ($P < 0.04$). These findings support the hypothesis that indomethacin reduces premature ovulation and cycle cancellation, therefore improving the efficacy of nIVF. In addition, the oocyte retrieval rate per procedure was not affected significantly by the use of indomethacin.

The embryo transfer rate was similar between the two groups studied, showing that indomethacin has no obvious effect on embryo development. The clinical pregnancy rate per cycle was increased in the indomethacin group but did not reach statistical significance which is possibly due to the small sample size. The clinical pregnancy rate per embryo transfer was not changed significantly by the use of indomethacin, as it did not affect embryo quality or implantation.

The clinical pregnancy rate per embryo transfer for women 35 years of age or less taking indomethacin was higher, but this was not statistically significant. On the other hand, women 35 years of age and younger had higher pregnancy rates, which is most probably due to better oocyte and embryo quality. This finding suggests that conventional IVF cycles may be a better option for women over the age of 35 years, as reported in a previous publication (Phillips et al., 2007). This is because in conventional IVF cycles, more embryos can be transferred to overcome the lower implantation rates per embryo transferred in this group of women. In addition, findings from this study reveal that the majority of pregnancies in nIVF occurred within three cycles. Therefore, further nIVF attempts would not be advisable after three failed nIVF cycles.

Furthermore, this study did not show any effect of indomethacin on implantation, which is supported by the findings of a study that looked at the effect of indomethacin on implantation in oocyte recipients (Bernabeu et al., 2006). Although no differences were noted in terms of pregnancy rate in the two groups of patients, it is important to note that further studies should look at any effects of NSAID at the follicular level, in particular oocyte and granulosa cell effects potentially caused by the interruption of prostaglandin action on the follicle. Furthermore, due to the suggested association of NSAID with miscarriage, it will be important to follow the pregnancies obtained from modified nIVF cycles using indomethacin to ensure that there is not an increased rate of miscarriage. (Nielsen et al. 2001). Thus far, the data (unpublished) do not suggest any increase in miscarriage rate.

### Table 2. Clinical pregnancy rates in the indomethacin treated versus no indomethacin groups.

<table>
<thead>
<tr>
<th>Clinical pregnancy rate</th>
<th>Indomethacin</th>
<th>No indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per cycle (%)</td>
<td>11/84 (13)</td>
<td>11/171 (6)</td>
</tr>
<tr>
<td>Per embryo transfer (%)</td>
<td>9/42 (21)</td>
<td>11/80 (14)</td>
</tr>
<tr>
<td>Per retrieval procedure (%)</td>
<td>11/79 (14)</td>
<td>11/143 (8)</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups.

### Table 3. Clinical pregnancy rate and natural IVF cycle attempt number.

<table>
<thead>
<tr>
<th>Natural IVF attempt</th>
<th>Clinical pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With indomethacin</td>
</tr>
<tr>
<td>1</td>
<td>9/11 (82)</td>
</tr>
<tr>
<td>2</td>
<td>2/11 (18)</td>
</tr>
<tr>
<td>3</td>
<td>–</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between the two groups.
A limitation of this study is its retrospective nature, which has inherent biases such as the selection of patients and observation bias. In addition, a multivariate logistic regression analysis was performed to overcome confounding variables with regard to the main outcomes. A prospective randomized controlled trial would be required to confirm findings obtained from this study.

In conclusion, so far as is known, this is the first study to show the impact of using GnRH antagonist in conjunction with indomethacin. Based on these data it has been demonstrated that the use of indomethacin significantly reduces premature ovulation before oocyte retrieval in modified nIVF. This is an interesting and potentially useful finding that demands further investigation.

Acknowledgements

The authors would like to thank all members of the OVO Fertility Clinic team for their support. The authors report no financial or commercial conflicts of interest.

References


Received 15 May 2007; refereed 5 July 2007; accepted 19 October 2007.
Appendix 4


Contribution by SJ Phillips
- Data collection
- Data analysis
- Manuscript writing and editing

Citation Metrics
- Google Scholar: 8
- Scopus: 5
- Web of Science: 4
Case report

Ongoing pregnancy after ICSI of frozen-thawed PESA-retrieved spermatozoa and IVF in a controlled natural cycle

Dr Kadoch completed his studies in medicine at the Saint-Antoine Faculty of Medicine (Université Paris VI, France) in 1995. After 5 years of residency in Obstetrics and Gynaecology in Paris, he was offered a fellowship position from 2000 to 2002 with Professor René Frydman (Clamart-France), where he acquired training in reproductive medicine and surgery. He spent 2 years as a Clinical Fellow in Reproductive Endocrinology and Infertility in the Centre Hospitalier de l’Université de Montréal (CHUM), and currently he is an Assistant Professor there, in the Obstetrics and Gynaecology department. Dr Kadoch has special interest in the development of new techniques such as natural cycle IVF and IVM to improve the quality of patient care.

Abstract

The recovery of a mature oocyte from a natural cycle followed by IVF (nIVF) is an attractive alternative to conventional IVF, involving ovarian stimulation, in the treatment of female infertility. Similarly, surgical recovery of spermatozoa from the epididymis by percutaneous sperm aspiration (PESA) has simplified the treatment of men with obstructive azoospermia. A couple sought treatment for diminished ovarian reserve and male factor infertility using IVF. A mature oocyte was retrieved and was inseminated by intracytoplasmic sperm injection (ICSI), following recovery of spermatozoa by PESA. A good quality embryo was transferred. A viable pregnancy was confirmed by ultrasound scan. A healthy baby boy was delivered naturally at 37 weeks gestation. This study reports the first ongoing clinical pregnancy and subsequent birth resulting from ICSI of spermatozoa retrieved by PESA into an oocyte recovered during a natural cycle. The use of a combination of less invasive assisted reproductive techniques (PESA and nIVF) can overcome barriers to fertility.

Keywords: ICSI, IVF, natural cycle, PESA

Introduction

The first successful IVF was performed in the natural cycle of an infertile woman with tubal factor infertility (Steptoe and Edwards, 1978).

Ovarian stimulation can increase oocyte and embryo numbers as well as pregnancy rates (Fischel et al., 1985). Hence, ovarian stimulation became a widely used method in the treatment of infertility, and natural IVF (nIVF) was soon abandoned in favour of stimulated IVF (sIVF). sIVF increases the probability of obtaining more than one fertilizable oocyte as well as that of conception. sIVF treatment requires ovarian follicular stimulation to increase the number of oocytes and the successful treatment outcome (Healy et al., 1994).

However, improvements in laboratory techniques and methods of follicular aspiration have created renewed interest in nIVF. Gonadotrophin-releasing hormone (GnRH) antagonists induce a reversible medical hypophysectomy, which prevents the occurrence of premature LH surges and thus increases the likely success of a cycle of nIVF (Rongieres-Bertrand et al., 1999).

IVF with intracytoplasmic sperm injection (ICSI) has been introduced as a potential treatment for severe male infertility, and high rates of fertilization and pregnancy have been reported (Palermo et al., 1992; Van Steirteghem et al., 1993). In the absence of spermatozoa in the ejaculate (azoospermia), the introduction of micro-epididymal and percutaneous epididymal sperm aspiration (PESA) followed by intracytoplasmic injection (ICSI) also appeared to be potential
breakthroughs for the treatment of severe male infertility (Shrivastav et al., 1994). The few drops of fluid obtained during a PESA procedure may contain sufficient spermatozoa to be frozen for several ICSI procedures.

**Case report**

A 35-year-old woman and her 43-year-old spouse were referred for assisted reproduction due to decreased ovarian reserve diagnosed by low antral follicle count on ultrasound examination and male infertility. Semen analysis revealed an ejaculate volume of 5.5 ml (normal >2 ml), a sperm concentration of 23.1 mol/ml (normal >20 mol/ml) and necrozoospermia.

The couple had had two children together previously and the male partner underwent an unsuccessful vasectomy reversal.

A baseline ultrasound scan was performed on day 3 of menses to exclude ovarian cysts and to ensure that the endometrial lining was <5 mm thick. Serial transvaginal ultrasounds were started on day 6. Follicular diameter was established by calculating the mean value of the two largest measurements perpendicular to each other. Subsequently, the patient was monitored until the leading follicle reached a diameter ≥14 mm. As soon as the dominant follicle reached a mean diameter of 14 mm, the patient received an antagonist (ganirelix, Orgalutran; Organon Pharmaceuticals, Scarborough, Ontario, Canada) (0.25 mg, s.c.) administered as an s.c. injection to avoid a spontaneous LH surge. Recombinant gonadotrophin (rFSH Puregon; Organon, 150 IU) was administered daily at the time of the first injection of antagonist to prevent a fall in the oestradiol concentration on the following day, and repeated thereafter until human chorionic gonadotrophin (HCG) administration. When the follicle had a diameter of ≥17 mm, the patient received HCG (Pregnyl; Organon Pharmaceuticals, 5000 IU) administered as an IM injection to achieve final follicular maturation. Transvaginal oocyte retrieval was scheduled 34–36 h after HCG administration, and produced a mature oocyte. Only the dominant follicle was aspirated.

Following retrieval of the oocyte, PESA was performed. A volume of 0.3 ml of fluid was obtained with a sperm count of 13.5 mol/ml and motility of 30%. Spermatozoa were washed in Cook fertilization media (Cook Canada, Toronto, Canada). The oocyte was assessed for maturity and then ICSI was performed according to standard protocols. The remaining sperm specimen was frozen for future use.

Fertilization was confirmed 18 h later with the presence of two pronuclei. Grading of the embryo was performed on day 2 after oocyte retrieval and before transfer. The embryo transferred on day 2 after oocyte retrieval was four cells, of good morphological quality with little fragmentation and equally sized blastomeres. Zona thinning was performed using the Hamilton Thorne ZILOS (Beverly, MA, USA). The transfer of the embryo to the recipient uterus was completed without complication on the first attempt. Unfortunately the patient did not conceive on this cycle. A second cycle was started from the patient’s next menses, following the same protocol. A single oocyte was retrieved, fertilized by ICSI using the frozen–thawed PESA sample. On day 2 after oocyte retrieval a 2-cell embryo with even blastomeres and no fragmentation was transferred. The luteal phase was supported by HCG (Pregnyl; Organon Pharmaceuticals) and vaginal natural P micronized progesterone (Prometrium; Schering, Pointe-Claire, Quebec, Canada) (600 mg daily) starting 2 days after oocyte retrieval and continued daily up to menstruation or for at least the first 8 weeks of pregnancy if the patient became pregnant. Serum HCG was collected 15 days after oocyte retrieval. The first ultrasound was performed at a gestational age of 6 weeks. A clinical pregnancy was defined as a pregnancy in which a fetal sac with fetal heart activity was visualized by ultrasound 8 weeks after HCG administration. A healthy baby boy was delivered at 37 weeks’ gestation.

**Discussion**

An ESHRE consensus meeting report recently stated that the essential aim of IVF/ICSI is the birth of one single healthy child, with a twin pregnancy being regarded as a complication (Land and Evers, 2003).

The collection of a mature oocyte from a naturally selected follicle, followed by IVF, has received increasing attention as an alternative to conventional sIVF treatment. The benefits of nIVF compared with sIVF include reduced cost (fewer drugs), reduced health risks (no hyperstimulation syndrome and multiple pregnancies), and increased patient acceptability (no blood tests). The financial benefits of nIVF as compared with sIVF have been reported previously (Daya et al., 1995). Although this protocol does require the patient to take some medication, it is greatly reduced as compared with normal stimulation protocols in sIVF, even mild controlled stimulation. The oocyte retrieval procedure with only one follicle is extremely quick and may be performed without anaesthesia or sedation. Since only one oocyte and therefore one embryo is obtained, there is no risk for multiple pregnancy.

So far as is known, this report describes the first ongoing clinical pregnancy and subsequent birth of a healthy baby resulting from ICSI of frozen–thawed PESA-retrieved spermatozoa and nIVF.

In summary, this study indicates that nIVF is a viable option for infertile women who have normal ovulatory menstrual cycles. If necessary, ICSI can be performed using spermatozoa retrieved by PESA. This report illustrates the use of a combination of fewer invasive assisted reproductive techniques in overcoming barriers to infertility. These results also indicate the importance of counselling regarding other possible options, such as sIVF, in cases such as this.

**Acknowledgements**

The authors would like to thank all members of the reproduction team for their support.

**References**


Fishel SB, Edwards RG, Pardy JM et al. 1985 Implantation, abortion, and birth after in vitro fertilization using the natural menstrual cycle or stimulation with clomiphene citrate and


Received 8 February 2005; refereed 22 February 2005; accepted 11 March 2005.
Appendix 5


Contribution by SJ Phillips

Concept
Data collection
Manuscript writing and editing

Citation Metrics

Google Scholar: 5
Scopus: 1
Web of Science: 1
Surgically retrieved spermatozoa versus ejaculated spermatozoa in modified natural IVF–ICSI cycles

Wael Jamal a,*, Maria P Vélez a,b, Armand Zini a,c, Simon Phillips a, Robert Hemmings a,d, Isaac-Jacques Kadocha,b

a OVO Clinic, 8000 boulevard Décarie, Montréal, Québec, Canada H4P 2S4; b Université de Montréal, Department of Obstetrics and Gynecology, Pavillon Roger-Gaudry, C.P. 6128, succursale Centre-Ville, Montréal, Québec, Canada H3C 3J7; c McGill University, Division of Urology, Department of Surgery, St. Mary's Hospital, 3830 Lacombe Ave., Montreal, Quebec, Canada H3T 1M5; d McGill University, Department of Obstetrics and Gynecology, St. Mary's Hospital, 3830 Lacombe Ave., Montreal, Quebec, Canada H3T 1M5

* Corresponding author. E-mail address: w.jamal@cliniqueovo.com (W Jamal).

Dr Wael Jamal received his BSc and MD in 1998 from the King Abdul-Aziz University in Saudi Arabia. He then undertook his obstetrics and gynaecology residency training at McGill University, a 1-year joint fellowship programme in minimally invasive gynaecological surgery between the University of Montreal and McGill University Health and a 2-year reproductive endocrinology and infertility fellowship programme between the University of Montreal and the OVO Clinic, completing in 2009. Afterwards, he joined the teaching faculty of the University of Montreal-affiliated reproductive endocrinology and infertility fellowship programme at the OVO Clinic. His particular research interests include the use of 3D ultrasound, sperm morphology and mild-stimulation IVF.

Abstract A retrospective cohort study was performed to evaluate the outcome of modified natural IVF–intracytoplasmic sperm injection (mnIVF–ICSI) cycles to compare 81 mnIVF–ICSI first cycles using ejaculated spermatozoa with 44 mnIVF–ICSI first cycles using surgically retrieved spermatozoa. There were no differences between the two groups in terms of number of oocytes retrieved, oocyte maturity or female age. However, male age was significantly higher in the surgically retrieved compared with the ejaculated group (41.5 versus 36.5 years, \(P = 0.001\)). There were no significant differences in fertilization rate or cleavage rate between the ejaculated and the surgically retrieved groups; however the prevalence of embryo transfer was higher in the surgically retrieved group (65.9% versus 45.7%, \(P = 0.03\)). Only single-embryo transfer was performed. Biochemical (34.5% versus 37.8%) and clinical (31.0% versus 35.1%) pregnancy rates per embryo transfer were similar between the ejaculated and the surgically retrieved groups. The data suggest that mnIVF–ICSI is an alternative treatment option in couples with severe male factor infertility where surgical sperm retrieval is required.

© 2012, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: ICSI, IVF, modified natural IVF, PESA, single-embryo transfer, TESE
Introduction

Modified natural cycle IVF (mnIVF) is a treatment modality that was of little interest for many years but has recently been used increasingly as an alternative option to stimulated IVF cycles (Aanesen et al., 2010; Reyffmann et al., 2007; Ubaldi et al., 2004). This change has been made possible with the introduction of gonadotrophin-releasing hormone (GnRH) antagonists and the development of techniques to minimize premature ovulation in these cycles (Kettel et al., 1991).

The benefits of mnIVF treatment are the use of minimal amount of medication with almost no risk of ovarian hyperstimulation syndrome, the relatively easier oocyte retrieval which can be done without sedation or local anaesthesia, the lower financial burdens and the possibility to continue with treatments on a monthly basis unlike stimulated IVF (sIVF) where a break from treatment is required (Aanesen et al., 2010; Pelinck et al., 2002). Furthermore, since mnIVF almost always results in only one oocyte being aspirated, single-embryo transfer will almost always be done, therefore reducing the risk of multiple birth (ESHRE, 2001; Pandian et al., 2005).

Although the clinical pregnancy rates per cycle and per embryo transfer in mnIVF may seem low, as reported in a literature review ranging from 0% to 18.8% and 0% to 50%, respectively (Pelinck et al., 2002), cumulative pregnancy rates with repeated mnIVF cycles, which are more accurate for assessing the efficacy of mnIVF are encouraging. Lifetime analysis from three published studies showed cumulative pregnancy rates of 43% and 42% after three and five oocyte aspirations (Aboulghar et al., 1995; Paulson et al., 1992) and 46% after four started cycles (Nargund et al., 2001). Furthermore, cancellation rates, the most important drawback of mnIVF, have decreased after the introduction of GnRH antagonists and indomethacin in the late follicular phase of mnIVF without negative impact on the clinical outcome (Paulson et al., 1994; Rongieres-Bertrand et al., 1999).

mnIVF has been proposed for various patient groups including poor responders (Kadoch et al., 2011; Reyffmann et al., 2007) and patients less than 37 years of age (Phillips et al., 2007). Additionally, it has been offered in the study centre as an alternative option to sIVF because of its lower cost for eligible couples when medically indicated. More recently, with the public funding of IVF in the province of Quebec, while up to three cycles of sIVF are covered by Medicare, up to six cycles of mnIVF, along with all its potential advantages, can be performed (Bissonnette et al., 2011). Indeed, some studies have reported that the cost of mnIVF is approximately 20% lower than that of sIVF, including average cost of cancelled cycles and the costs related to ovarian hyperstimulation syndrome (Aboulghar et al., 1995; Nargund et al., 2001). More recently, Polinder et al. (2008) conducted a randomized controlled trial evaluating the cost-effectiveness of mild ovarian stimulation/GnRH antagonist co-treatment combined with single-embryo transfer, compared with a standard stimulation/GnRH agonist long protocol and the transfer of two embryos. The authors reported that despite a significantly increased average number of IVF cycles using the mild strategy (2.3 versus 1.7 cycles), lower average total costs were observed over a 12-month period. This was mainly due to higher costs of the obstetric and post-natal period for the standard strategy, related to multiple pregnancies.

There is limited information on the use of mnIVF in male factor infertility. The use of intracytoplasmic sperm injection (ICSI) in sIVF protocols for the treatment of male infertility has benefited many couples (Palermo et al., 1992). Both ejaculate spermatozoa and those retrieved from the testis (testicular sperm extraction, TESE) and epididymis (percutaneous epididymal sperm aspiration, PESA) have been utilized in insemination of oocytes using ICSI (Craft and Shrivastav, 1994; Temple-Smith et al., 1985). This study centre published a case report several years ago reporting the use of surgically retrieved spermatozoa in combination with mnIVF and the successful birth of a healthy boy (Kadoch et al., 2005). Following that success, the objective of the present study was to evaluate the ongoing results of mnIVF in combination with surgical sperm retrieval in this clinic.

Materials and methods

mnIVF and ICSI cycles in women under 37 years of age performed at OVO Clinic between December 2003 and September 2011 were included in this retrospective cohort study. Only first cycles that underwent oocyte retrieval were included in the analysis. The study recruited couples with mild-to-moderate male factor (total motile sperm count, TMC, 1–5 million in the pre-ICSI evaluation) with an indication for IVF–ICSI. The study excluded men with severe male factor infertility (<1 million in the pre-ICSI evaluation) or mild male factor infertility (>5 million in the pre-ICSI evaluation). On the day of ICSI, only cycles with mild-to-moderate ICSI indication (motile spermatozoa between 0.1–5 million on IVF day) were included in the study (Figure 1). Patients were not excluded if their TMC dropped below 1 million (but remained above 0.1 million) on the day of ICSI as this would have resulted in a substantial drop in the total number of cases. It was felt that couples with a drop in their TMC (on the day of ICSI) in the range of 0.1 to 1 million would have similar outcomes to couples with a TMC between 1 and 5 million. Couples with diagnosis of unexplained infertility, tubal factor, low ovarian reserve, endometriosis, sperm donor or recurrent miscarriage were excluded from the study (Figure 1).

The mnIVF protocol has been described previously (Kadoch et al., 2008, 2011; Phillips et al., 2007). Patients were seen on day 9 of their cycle for an initial ultrasound. Follicular development was monitored and GnRH antagonist (ganirelix, Orgalutran; Merck Frosst, Kirkland, Quebec, Canada) 0.25 mg s.c. was commenced when the dominant follicle reached 14 mm; at the same time 50 mg indomethacin (Indocid; Merck Frosst) was given three times a day until the day before oocyte retrieval to reduce premature ovulation. The use of indomethacin, a cyclooxygenase inhibitor, has been associated with delayed follicular rupture, reducing premature ovulation (Athanasiou et al., 1996; Kadoch et al., 2008; Morris et al., 2007; Nargund et al., 2001). Additionally, 150 IU human menopausal gonadotrophin (HMG)
(Repronex, Ferring Canada, North York, Ontario, Canada) were prescribed to counteract the oestradiol drop associated with GnRH antagonist use. The three medications are started together and administered daily (average 2.7 days) until the day of human chorionic gonadotrophin (HCG) trigger, which is administered once the follicle attains 17–18 mm (5000 IU, Pregnyl; Merck Frosst). Transvaginal oocyte retrieval was performed 34 h later.

Semen samples were produced by the male partner immediately after oocyte retrieval into a sterile, sperm-tested container. Semen was assessed at 37°C after 30 min and prepared using density gradients of Puresperm (Nidacon, Sweden) and washed with Gamete Buffer (Cook, Canada).

Spermatozoa from men with obstructive azoospermia were surgically retrieved by PESA or TESE prior to the ovarian stimulation cycle. PESA samples were obtained several weeks prior to the IVF cycle, cryopreserved using Sperm Maintenance Media (Irvine, Somagen, Canada) and subsequently thawed on the day of oocyte retrieval. In cases where the PESA sample was suboptimal (e.g., rare or nonmotile spermatozoa) or could not be performed prior to the ICSI cycle, TESE was performed 24–48 h prior to oocyte retrieval to permit culture of the tissue at 37°C (Morris et al., 2007).

At oocyte retrieval, the oocyte was collected into Cook Fertilization media (Cook), and prepared for ICSI by denudation of cumulus cells using cumulase (Origio, New Jersey USA). Assessment of maturity for ICSI at metaphase II was assessed by noting extrusion of the first polar body. In addition, birefringence technology was used to assess spindle presence and location using a polscope (Oosight; CRi, USA). Fertilization was confirmed by assessment of two pronuclei 18 h post-sperm injection and embryo transfer was performed on day 2.

Biochemical pregnancy was assessed by serum hHCG concentration 15 days post-embryo transfer (hHCG higher than 25 IU) and confirmed by ultrasound evidence of an intrauterine fetal heart at 8 weeks (clinical pregnancy). Live birth rates were calculated for those patients with estimated date of delivery before October 2011.

Statistical analysis was based on the assumption that clinical pregnancy rates per embryo transfer in mnIVF–ICSI cycles are approximately 30% and that the difference between both groups of sperm source will be less than 25%.
Sperm source in natural-cycle IVF

A total of 726 first mIVF cycles were performed in the study centre between December 2003 and September 2011. Ninety-two (12%) were cancelled before oocyte retrieval. In the 634 cycles that underwent oocyte retrieval, at least one oocyte was recovered in 553 cycles: an oocyte recovery rate of 87.2% (Blackwelder, 1982).

After excluding the noneligible couples, (Figure 1) 81 first mIVF–ICSI cycles utilizing ejaculated spermatozoa were compared with 44 cycles using surgically retrieved spermatozoa (PESA, 30 cycles; TESE, 14 cycles). The ejaculated sperm group comprised patients with mild-to-moderate ICSI indication (mobile spermatozoa between 0.1–5 million on IVF day; mean ± SD 1.41 million ± 1.40 million). All the patients in the surgically retrieved sperm group had a diagnosis of obstructive azoospermia (prior vasectomy, vasectomy reversal failure or congenital bilateral absent vas deferens), except one with presumed obstructive azoospermia (with a normal exam and serum FSH, and a normal karyotype and no Y chromosome microdeletion).

The descriptive characteristics of the two groups can be seen in Table 1. There were no differences between the two groups in terms of fertilization rate or cleavage rate between the ejaculated and surgically retrieved sperm groups. However, higher rates of embryo transfer were found in the surgically retrieved sperm group. Single-embryo transfer was performed in all cases. There were no differences in the biochemical or the clinical pregnancy rate. Of note, all ongoing clinical pregnancies at the 8-week ultrasound were singleton pregnancies.

A few authors have evaluated the outcomes of mIVF and ICSI using ejaculated spermatozoa in cases of male factor infertility. Ubaldi et al. (2004) assessed 157 couples with severe male factor infertility and poor ovarian response who underwent a total of 258 natural ICSI cycles. Thirty-two cycles were cancelled before oocyte retrieval. In the 226 cycles that underwent oocyte retrieval, at least one oocyte was recovered in 193 cycles with an oocyte recovery rate of 85.4%. The authors were able to perform an embryo transfer in only 51.5% (133/258) of the started cycles. In the current study, however, the proportion of embryo transfer was lower in the ejaculated group (45.7%) and higher in the surgically retrieved sperm group (65.9%), respectively. Ubaldi et al. (2004) reported clinical pregnancy rates of 22.2% per patient and 26.3% per embryo transfer. The current study obtained similar results, with clinical pregnancy rates

Table 1  Demographic comparison between ejaculated spermatozoa and surgically retrieved spermatozoa.

<table>
<thead>
<tr>
<th></th>
<th>Ejaculation (81 cycles)</th>
<th>Surgical retrieval (44 cycles)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female age (years)</td>
<td>31.7 ± 3.2</td>
<td>32.0 ± 3.2</td>
<td>NS⁵</td>
</tr>
<tr>
<td>Male age (years)</td>
<td>36.5 ± 6.2</td>
<td>41.5 ± 8.3</td>
<td>0.001⁵</td>
</tr>
<tr>
<td>No. of oocytes collected</td>
<td>1.06 ± 0.29</td>
<td>1.02 ± 0.15</td>
<td>NS⁵</td>
</tr>
<tr>
<td>Oocyte maturity</td>
<td>94 (80/85)</td>
<td>100 (45/45)</td>
<td>NS⁵</td>
</tr>
</tbody>
</table>

Data are mean ± SD or % (n/total oocytes).
NS = not statistically significant.
⁴t-test equal variances.⁵t-test unequal variances.
⁶Fisher Exact test.
of 16.0% and 20.5% per oocyte retrieval and 35.1% and 31.0% per embryo transfer in the ejaculated and the surgically retrieved groups, respectively. These results are comparable to studies of poor responders (Bassil et al., 1999) and normally responding younger women (Janssens et al., 2000; Ng et al., 2001).

Recently, Jones and Liu (2011) published a case report of a 35-year-old woman with significantly elevated FSH concentrations, regular menses and severe male factor infertility who conceived after her third mnIVF attempt with ICSI, recommending mnIVF as an option prior to considering oocyte donation when a secondary cause of infertility necessitates ICSI. In this study centre’s previously published case report, a 35-year-old woman and her 43-year-old spouse diagnosed with secondary infertility were referred for assisted reproduction due to decreased ovarian reserve and male factor infertility with unsuccessful vasectomy reversal (Kadoch et al., 2005). Following the previously described mnIVF protocol, oocyte retrieval was performed and PESA was conducted thereafter. Transfer of a good-quality embryo was accomplished in this first cycle; however, no pregnancy was achieved. Subsequently, a second mnIVF cycle was performed with successful oocyte retrieval and fertilization using a sample of the remaining frozen spermatozoa obtained with PESA. As result of this second mnIVF cycle, a healthy baby boy was delivered at 37 weeks’ gestation. Supported by this initial case report, it was suggested considering mnIVF as an alternative option for infertile women who have normal ovolatory menstrual cycles; and when necessary, ICSI could be performed using spermatozoa retrieved by PESA (Kadoch et al., 2005).

In most studies to date, the source of spermatozoa (ejaculated, epididymal or testicular) used in ICSI cycles has had no significant impact on clinical outcomes and this is also true within the context of single-embryo transfer (Nagy et al., 1995; Nilsson et al., 2007). Moreover, there is no difference in reproductive outcomes whether fresh or frozen spermatozoa are used in ICSI (Nagy et al., 1995). Nonetheless, the mnIVF represents a unique IVF scenario in that a single oocyte is obtained and a single spermatozoon is used. Although there are no data to show even a modest impact of sperm source on ICSI outcomes, if there were such an effect it would potentially be best demonstrated in the context of a single oocyte injection (as in a mnIVF). Therefore, this study assumed that the use of a single, surgically retrieved spermatozoa (i.e. epididymal or testicular) would adversely impact reproductive outcomes when compared with the use of a single ejaculated spermatozoa. The fact that this study observed no effect of sperm source on mnIVF outcomes further supports the premise that the source of spermatozoa has no significant impact on ICSI reproductive outcomes in general.

The primary limitation of this study is its relatively small sample size. This limited its ability to adjust for a broad range of potential covariates, including female factors, although the selection of women younger than 37 years would decrease confounding by female age. Although the sample size is large enough to indicate that the use of surgically retrieved spermatozoa does not have an important and significant adverse impact on reproductive outcomes following mnIVF (i.e. >25% reduction in clinical pregnancy rates), we cannot exclude the possibility that the use of surgically retrieved spermatozoa could have a more modest adverse effect on clinical outcomes.

Additionally, while only first mnIVF cycles were included in this study, some couples had previously undergone stimulated cycles at the study clinic or in another institution. In fact, while most of the patients (78.5%) did not have a prior stimulated cycle, 15.6% had one and 5.9% had between three and six previous stimulated cycles (data not shown). Nonetheless, since the distribution was similar in both groups, if this would introduce a bias, it would not have an impact on the interpretation of the results.

These results allow the conclusion that, for couples with obstructive azoospermia, mnIVF could be a reasonable alternative to conventional sIVF where occasionally one may have a larger number of oocytes than spermatozoa

### Table 2  Comparison of outcome measures between ejaculated spermatozoa and surgically retrieved spermatozoa.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Ejaculation (81 cycles)</th>
<th>Surgical retrieval (44 cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilization rate</td>
<td>48 (59.3)</td>
<td>30 (68.2)</td>
</tr>
<tr>
<td>Cleavage rate</td>
<td>43 (53.1)</td>
<td>30 (68.2)</td>
</tr>
<tr>
<td>Cycles with embryo transfer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37 (45.7)</td>
<td>29 (65.9)</td>
</tr>
<tr>
<td>Cycles with assisted hatching</td>
<td>15 (18.5)</td>
<td>9 (20.5)</td>
</tr>
<tr>
<td>Biochemical pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per oocyte retrieval</td>
<td>14/81 (17.3)</td>
<td>10/44 (22.7)</td>
</tr>
<tr>
<td>Per embryo transfer</td>
<td>14/37 (37.8)</td>
<td>10/29 (34.5)</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per oocyte retrieval</td>
<td>13/81 (16.0)</td>
<td>9/44 (20.5)</td>
</tr>
<tr>
<td>Per embryo transfer</td>
<td>13/37 (35.1)</td>
<td>9/29 (31.0)</td>
</tr>
</tbody>
</table>

Data are n (% per cycle) or n/total (%).
<sup>a</sup>Significantly higher for surgical retrieval (P = 0.03, t-test).
for the IVF cycle. mnIVF may be considered in couples where the man has had a vasectomy, if the partner is eligible for mnIVF. The data demonstrates that the need to surgically retrieve spermatozoa should not be a contraindication to using mnIVF.

Acknowledgements

The authors would like to thank the clinical and laboratory teams at OVO Clinic for all their work on these cycles. MPV is supported by a CIHR fellowship award.

References


Declaration: The authors report no financial or commercial conflicts of interest.

Received 4 February 2012; refereed 6 June 2012; accepted 12 June 2012.
Appendix 6

Kadoch IJ, Phillips SJ, Bissonnette F. Modified natural cycle in vitro fertilization should be considered as the first approach in young poor responders. Fertility and Sterility. 2011. Vol 96(5) :1066-1068

Contribution by SJ Phillips

Concept

Manuscript writing and editing

Citation Metrics

Google Scholar: 20
Scopus: 13
Modified natural-cycle in vitro fertilization should be considered as the first approach in young poor responders

Isaac-Jacques Kadoch, M.D., Simon J. Phillips, M.Sc., and François Bissonnette, M.D.

The use of modified natural-cycle in vitro fertilization (IVF) is a valuable alternative to controlled ovarian hyperstimulation in young poor responders and should be considered in patients who require IVF and demonstrate endocrinologic evidence of ovarian aging and in those who have had one or two canceled controlled ovarian hyperstimulation cycles. (Fertil Steril® 2011;96:1066–8. ©2011 by American Society for Reproductive Medicine.)

Key Words: In vitro fertilization, natural cycle, poor responders

Although the first pregnancy obtained by in vitro fertilization (IVF) with embryo transfer was achieved with a natural cycle—and indeed natural-cycle IVF was the method of choice for the first few years of IVF treatment (1)—this procedure was soon abandoned in favor of gonadotropin-stimulated protocols in controlled ovarian hyperstimulation (COH). The use of COH allowed an increase in the number of recruited oocytes and an increase in the pregnancy rates associated with IVF (2). It also has permitted excess embryos to be cryopreserved in the event that the IVF attempt fails to result in a pregnancy or when the patient desires additional sibling attempts without a need for further ovarian stimulation. However, due to the additional costs, risks, and complexity of ovarian stimulation, both the patient and the caregiver feel the pressure to increase the chances of the patient becoming pregnant; therefore, it has become standard to transfer more than one embryo.

In some patients, the induction of multiple follicular growth is not achieved with IVF in a stimulated cycle. Diminished ovarian reserve is the main reason for poor ovarian response in these patients. Some tests are considered as primary markers of poor ovarian response: an increased serum baseline follicle-stimulating hormone (FSH) level, a decreased value of antimüllerian hormone (AMH), or a significant reduction in the antral follicle count.

Over time, oocyte quality decreases in parallel with progressive follicle loss. The combination of decreases in both the quality and number of oocytes explains declining fecundity in females. The diminution of follicle count in women younger than their midthirties is the main limiting factor, and quality is preserved.

In a natural cycle, several follicles are recruited initially, but it is only one that attains dominance and goes on to ovulate. Being able to control ovulation was one of the reasons why natural-cycle IVF was replaced with stimulated-cycle IVF; the first oocyte retrievals had to be performed according to the natural leuteinizing hormone (LH) surge, which resulted in collecting the oocyte at any time of the day or night before ovulation.

A mounting interest in natural-cycle IVF has challenged the medical community to better understand the mechanisms controlling the follicular phase and ovulation in particular, in an effort to optimize this procedure and its outcome. Improvements in laboratory techniques and methods of follicular aspiration have created renewed interest in natural-cycle IVF. Gonadotropin-releasing hormone (GnRH) antagonists induce a reversible medical hypophysectomy, which prevents the occurrence of premature LH surges and thus increases the likely success of a cycle of natural-cycle IVF (3). Indomethacin use during the late follicular phase has also been shown to decrease the rate of spontaneous ovulation and hence provide a higher oocyte retrieval success rate in modified natural-cycle IVF (4).

In fact, the use of modified natural-cycle IVF minimizes physical and emotional stress for the patient and significantly reduces the cost of drugs and laboratory tests for the assisted reproduction unit. Although modified natural-cycle IVF does not reach the levels of pregnancy rate that can be obtained by transferring multiple embryos after ovarian stimulation, we believe that it can be a useful, easy, and cheap tool in the treatment of young poor responders. The benefits of modified natural-cycle IVF are obvious: the increasing problem of multiple pregnancy is removed, the patients do not need to inject themselves with large quantities of expensive medications, and the less invasive nature of the procedure (no sedation or anesthesia is used for the oocyte collections) allows patients to have a cycle each consecutive month. Therefore, as with intrauterine insemination, patients can have three cycles of modified natural-cycle IVF consecutively with minimal impact on their life schedules.

In our center, modified natural-cycle IVF protocol is performed as follows. A baseline transvaginal ultrasound scan is performed on day 3 of menses to exclude ovarian cysts and to ensure that the endometrial lining is <5 mm thick. Serial ultrasound examinations are started on day 6. The follicular diameter is established by Voluson E8, calculating automatically the mean value. Subsequently, the patient is monitored until the leading follicle reaches a diameter >14 mm. Clinicians, therefore, use surrogate markers of follicular maturation such as estrogen production and follicular size. As soon as the dominant follicle reaches a mean diameter of 15 mm and the endometrium a thickness of 6 mm, the patient receives 0.25 mg SC of GnRH antagonist (Ganirelix, Orgalutran; Merck Canada). Indomethacin (Indocid; Merck Frosst) at a dosage of 50 mg orally three times a day is also started on the same day to prevent spontaneous ovulation. Both are continued until the human chorionic gonadotropin (hCG) is administered. Human menopausal gonadotropin (hMG, Repronex; Ferring Canada), 150 IU
SC, is administered daily at the time of the first injection of GnRH antagonist to prevent a fall in the estradiol concentration on the following day, and it is repeated thereafter until hCG administration. When the follicle has a diameter of 17 mm, the patient receives 5,000 IU IM of hCG (Pregnyl; Merck Canada) to achieve final follicular maturation. Transvaginal oocyte retrieval is scheduled 34 hours after hCG administration.

Modified natural-cycle IVF has recently received renewed attention and has been used for patients who have shown a poor response in previous attempts with COH. It has been suggested in recent years that natural-cycle IVF may be a promising alternative for poor responders (5, 6). The biological advantages of natural-cycle IVF may provide a single oocyte of better quality and thus allow the transfer of a healthier embryo into a more receptive endometrial environment (7). Overall, these data suggest that poor responders may benefit from natural-cycle IVF, and it should be offered in those younger patients with a diminished ovarian reserve. In our clinical experience, modified natural-cycle IVF should be considered as a first approach and not as a consequence of a previous treatment failure. Unfortunately, modified natural-cycle IVF is rarely proposed and only then as a last chance after previous standard attempts have failed. Reasonable pregnancy rates have been reported in several prospective studies where modified natural-cycle IVF was performed in poor responder patients (8–10). Although no large controlled prospective studies are available, we achieve a relatively satisfactory pregnancy rate per cycle compared with studies focused on poor responder patients undergoing different protocols for multiple ovulation induction. Better embryo quality as a consequence of natural oocyte selection, better endometrium receptivity, and monthly repeatability of the procedure can balance the relatively low chance of obtaining an embryo transfer.

For practical reasons, the advancement of the follicular phase in the menstrual cycle is commonly timed according to the onset of last menses. Shorter cycles in poor responders can be explained by the intercycle basal FSH signal. Efforts should be diverted toward controlling when it takes place, either with exogenous estrogen or with oral contraceptives (11). Practically, the prescription of ethinyl estradiol, 4 mg orally, during the 2 or 3 first days of the menstrual cycle can be useful in these conditions to delay follicular maturation and to synchronize with the endometrium.

While laboratory researchers are trying to find the best method to select an embryo or embryos with the best developmental potential for embryo transfer, perhaps the role of natural selection of follicles should be considered. Further research is necessary to verify whether the aneuploidy rate or gene expression of cumulus cells in natural-cycle embryos are improved over embryos resulting from stimulation cycles.

Because there is only one follicle in natural-cycle IVF, there is a greater risk of cycle cancellation than in stimulated cycles, even in younger patients. This is an area of natural-cycle IVF that needs more attention and research, as there is certainly less chance of an embryo transfer after the commencement of a natural-cycle IVF cycle when compared with a stimulated IVF cycle.

The number of women over 35 years of age attempting to conceive has increased significantly in the last decade. Although it is well established that a woman’s reproductive success dramatically declines with age, the underlying physiologic changes responsible for this phenomenon are not well understood. Many studies show that the risk of childlessness increases at higher ages as a consequence of ovarian aging. It is well known that the considerable increase in aneuploidy in embryos from older women contributes to these women’s inability to conceive, increasing both implantation loss and pregnancy failure (12, 13). Many studies have shown that oocyte quality has the greater impact on the reduction of fertility than oocyte number. The concept of using natural-cycle IVF for older patients has been previously suggested and has had very poor outcomes (14).

Because the pregnancy rate from modified natural-cycle IVF is lower (15), it appears that the benefits of transferring more than one embryo are increased in patients over the age of 35 years. Furthermore, in older patients, achievement of multiple follicle growth remains a challenge. Indeed, poor ovarian responses are commonplace after the age of 35 years in any assisted reproduction unit. The management of these patients is a challenge, and whatever protocol is used their clinical outcome remains poor compared with both young patients and those with normal response. The overall pregnancy rates achieved with modified natural-cycle IVF in older patients are low, and they are reduced with the transfer of a single embryo.

Apart from oocyte donation, there is no established intervention to improve the pregnancy outcome of older patients. Although the factors responsible for diminished oocyte quality remain to be elucidated, recent data focus on the potential role of mitochondria dysfunction in reproductive aging (16). In this context, the use of coenzyme Q10 as a mitochondria energy substratum has been proposed to reduce the aneuploidy rate and enhance the prognosis of modified natural-cycle IVF in older poor responders. This is an area currently under investigation in our clinic’s program.

The use of modified natural-cycle IVF is a valuable alternative to COH in young poor responders and is in line with the increasing interest in single-embryo transfer and the resultant reduction in multiple pregnancies. This alternative should be considered in patients who require IVF, who demonstrate endocrinologic evidence of ovarian aging, and who have had one or two canceled COH cycles. Hence, we suggest that modified natural-cycle IVF should be considered as a first approach in young poor responders. Furthermore, the increasing interest in the concept of the potential rejuvenation of ovarian reserve in mammalian females poses the question as to whether modified natural-cycle IVF could become a future option for older patients who have reduced ovarian reserve.

**REFERENCES**

Appendix 7


**Contribution by SJ Phillips**

- Data collection
- Manuscript writing and editing

**Citation Metrics**

- Google Scholar: 4
- Scopus: 4
- Web of Science: 3
Case report

Successful pregnancy in an ovarian agenesis patient after modified natural cycle IVF oocyte donation

Professor Kadoch is the Director of the Reproductive Endocrinology and Infertility Programme at the Department of Obstetrics and Gynaecology of the University of Montreal. He received his medical degree from the Saint-Antoine Faculty of Medicine in Paris, before preparing for Concours de l’Internat in 1995. He completed his residency in Obstetrics and Gynaecology in Paris. Then he spent more than 2 years in the service of Professor René Frydman in Clamart, acquiring extensive training in reproductive medicine and surgery. He also taught at the Faculty of Medicine of Paris. He has authored and co-authored numerous publications and mentors post-graduate students.

Professor Isaac Jacques Kadoch

Isaac Jacques Kadoch1,2, Wael Jamal1,2, Simon J Phillips2, Robert Hemmings2, Louise Lapensée1,2, Bernard Couturier2, François Bissonnette1,2

1University of Montreal, Department of Obstetrics and Gynecology, Saint Luc Hospital (CHUM), 1058 rue Saint-Denis, Montreal, Québec, Canada, H2X 3J4; 2Ovo Fertility, 8000 Décarie Boulevard, Suite 100, Montreal, Québec, Canada, H4P 2S4

3Correspondence: e-mail: ij.kadoch@umontreal.ca

Abstract

The recovery of a mature oocyte from a modified natural cycle followed by in-vitro fertilization (nIVF) is an attractive alternative to conventional IVF, involving ovarian stimulation, in the treatment of female infertility. Ovarian agenesis is a rare disorder resulting in primary amenorrhoea and infertility in affected females. A couple sought help for infertility due to ovarian agenesis of the female partner and decided to pursue treatment utilizing oocyte donation. Modified natural-cycle egg retrieval was carried out on the donor; one mature oocyte was retrieved and underwent IVF using a sperm sample from the male partner. A good-quality embryo was transferred. A viable pregnancy was confirmed by ultrasound scan and resulted in the delivery of a healthy baby boy at 36 weeks’ gestation. This is the second published report of an ongoing clinical pregnancy and subsequent birth resulting from oocyte donation recovered during a modified natural cycle. The use of less invasive assisted reproduction techniques such as nIVF can be used in oocyte donation cycles successfully.

Keywords: egg donation, IVF, modified natural cycle IVF, natural cycle, oocyte donation, ovarian agenesis

Introduction

The first successful IVF was performed in the natural cycle of an infertile woman with tubal factor infertility (Steptoe and Edwards, 1978). Ovarian stimulation can increase oocyte and embryo numbers as well as pregnancy rates (Fishel et al., 1985). Hence, ovarian stimulation became a widely used method in the treatment of infertility, and natural IVF was soon abandoned in favour of conventional (stimulated) IVF. Conventional IVF increases the probability of obtaining more than one fertilizable oocyte as well as that of conception and requires ovarian follicular stimulation to increase the number of oocytes and improve treatment outcome (Healy et al., 1994).

However, improvements in laboratory techniques and methods of follicular aspiration have created renewed interest in natural IVF. Gonadotrophin-releasing hormone (GnRH) antagonists induce a reversible medical hypophysectomy, which prevents the occurrence of premature LH surges and thus increases the likely success of a cycle of natural IVF (Rongieres-Bertrand et al., 1999). Indomethacin use during the late follicular phase has also been shown to decrease the rate of spontaneous ovulation and hence higher oocyte retrieval success rate in modified natural IVF cycles (Kadoch et al., 2008).

The clinical pregnancy rate per embryo transfer following modified natural IVF was reported to be 27% compared with 47% in conventional IVF cycles for patients aged less than 35 years old (Phillips et al., 2007). However, natural-cycle patients could attempt consecutive cycles with much less medical and financial
impact on their lives and in this age group the choice of modified natural IVF reduces the cost and risk to the patient, permitting her to have multiple consecutive attempts and offers a cumulative clinical pregnancy rate which approaches that of conventional IVF (Pelinic et al., 2007; Phillips et al., 2007).

Gonadal agenesis with a 46,XX chromosome complement is an extremely rare disorder affecting females with only 11 cases reported in the literature; 10 of them were discussed in a report by Mutchinick et al. (2005) and one was recently published by Dede et al. (2008). Out of those reported cases; only eight of them reached the age of puberty and exhibited primary amenorrhoea, lack of secondary sexual development and hypergonadotropic hypogonadism. Variable forms of Müllerian anomalies were observed in all of these patients. Treatment of these cases would involve administration of hormone replacement therapy in the form of oestrogen to achieve puberty along with secondary sexual development and maximum potential bone growth, in addition to progesterone to oppose the oestrogenic effect on the uterus, if present, and induce menstruation.

Case report

This report describes the case of a 38-year-old female and her 38-year-old partner who presented to the fertility centre for treatment. The female partner was a known case of ovarian agenesis which was diagnosed during her pubertal years when she presented with primary amenorrhoea and lack of secondary sexual development. She was found to have a normal 46,XX karyotype, absent ovaries and present normal Müllerian organs (vagina, cervix, uterus and tubes) and had been started on oestrogen/progesterone hormone replacement therapy which was later changed to combined oral contraceptive for the last 15 years. The patient experienced normal withdrawal menstrual bleeding. The patient was otherwise healthy with no history of any other medical or surgical disease and she didn’t use any other medication. On physical examination she had normal secondary sexual characteristics including normal vagina, cervix and palpable uterus. The male partner was a non-smoker and healthy with no history of any medical or surgical disease or the use of any medication. He had not previously fathered any pregnancies in the past.

The couple was seeking advice on fertility treatment using oocyte donation and the female partner’s sister had offered to donate to her sister. Basic work-up for the couple included viral serology (hepatitis B, hepatitis C, cytomegalovirus [CMV], human T-lymphocyte virus types I and II, and human immunodeficiency virus types I and II) and results were negative except for CMV immunoglobulin G, which was positive for both partners. Venereal Disease Research Laboratory (VDRL) and Chlamydia cultures were negative for the couple and the female was immune to rubella and had a normal cervical smear. Semen analysis for the male partner was normal.

The donor was a healthy, married 35-year-old, with two previous spontaneous pregnancies and the delivery of two healthy children. She had no history of any medical or surgical disease and has a regular menstrual cycle. Her hormonal workup indicated a normal profile including a day-3 FSH 6.6 IU/l, oestradiol 73 pmol/l and baseline day-3 transvaginal ultrasound study (TV-US), which showed a normal ovarian reserve with an antral follicle count of 16. VDRL and cervical swab for chlamydia cultures were negative, cervical smear was normal; the patient was rubella immune and had a negative viral serology for the same profile mentioned above.

A meeting with a psychologist was carried out for the couple and the donor, separately and together on different occasions. The female partner expressed her wish to minimize the risks and side effects of conventional IVF on the donor as well as the costs of medications, so modified natural IVF was offered as a plausible alternative to pursue the treatment. Informed consent was obtained from all participants in the cycle.

Modified natural IVF protocol was carried out on the donor as follows. A baseline TV-US was performed on day 3 of menses to exclude ovarian cysts and to ensure that the endometrial lining was <5 mm thick. Serial TV-US were started on day 6. Follicular diameter was established by calculating the mean value of the two largest measurements perpendicular to each other. Subsequently, the donor was monitored until the leading follicle reached a diameter >14 mm. As soon as the dominant follicle reached a mean diameter of 14 mm (day 11 of donor’s cycle), the donor received 0.25 mg, s.c. GnRH antagonist (Ganirelix, Orgalutran; Schering-Plough Canada, Pointe-Claire, Québec) administered to avoid a spontaneous LH surge. Indomethacin (Indocid; Merck Frosst, Kirkland, Québec) at a dose of 50 mg p.o. three times a day was also started on the same day to prevent spontaneous ovulation and both would be continued until human chorionic gonadotrophin (HCG) administration. Recombinant gonadotrophin (rFSH, Puregon; Schering-Plough Canada) 150 IU s.c. was administered daily at the time of the first injection of GnRH antagonist to prevent a fall in the oestradiol concentration on the following day, and repeated thereafter until HCG administration. When the follicle had a diameter of 17 mm (day 13 of donor’s cycle), the donor received 5000 IU i.m. HCG (Pregnyl; Schering-Plough Canada) administered as to achieve final follicular maturation. Transvaginal oocyte retrieval was scheduled 34–36 h after HCG administration (day 15 of donor’s cycle) and produced a mature oocyte. Only the dominant follicle was aspirated.

The recipient protocol at the study centre involved the use of a GnRH agonist (nafarelin acetate; Synarel, Pfizer Canada, Kirkland, Québec) 1 puff intranasally twice daily along with micronized 17β-oestradiol (Estrace; Shire BioChem Inc., Saint Laurent, Québec) 2 mg p.o. and 2 mg p.v. once daily and aspirin (Asaphen EC; Pharmascience, Montréal, Québec) 81 mg p.o. once daily. The GnRH agonist was used in the recipient protocol to avoid any possibility of LH triggering caused by the continuous daily use of micronized 17β-oestradiol.

This protocol was followed for 12 days after which the GnRH agonist was stopped, a TV-US was performed to assess endometrial thickness (7.7 mm at that point) and then the recipient continued with Estrace and aspirin until the day of HCG injection of the donor when a second TV-US was performed to assess endometrial thickness and ensure that it was at least 8 mm. In this case the recipient responded well to the protocol and her endometrial thickness was at 10 mm. Luteal support on the recipient was started on the same day of HCG injection of the donor in the form of micronized progesterone (Prometrium; Schering-Plough Canada) 300 mg p.v. twice daily, and would continue along with Estrace and aspirin for 12 weeks. In addition for luteal support, HCG 2500 IU s.c. (Profasi; EMD Serono)
Canada, Mississauga, Ontario) was administered to the recipient once every other day for a total of three doses starting on the same day as the HCG injection of the donor. The recipient also took ciprofloxacin 250 mg p.o. twice daily for 5 days; starting on the day of HCG injection of the donor.

A single oocyte was retrieved from the donor and was inseminated using a washed sperm sample from the male partner. Fertilization was confirmed 18 h later by the presence of two pronuclei and two polar bodies. The embryo cleaved 24 h later, resulting in a good-quality embryo, which was transferred on day 2 as a 5-cell grade II embryo. The embryo transfer was uneventful. The patient had a positive serum pregnancy test 2 weeks after embryo transfer and polar bodies. The embryo cleaved 24 h later, resulting in a good-quality embryo, which was transferred on day 2 as a 5-cell grade II embryo. The embryo transfer was uneventful. The patient had a positive serum pregnancy test 2 weeks after embryo transfer and polar bodies.

The pregnancy progressed uneventfully, and the patient gave birth at gestational age of 36 weeks to a healthy male fetus of 2.46 kg. Both mother and baby are in excellent condition.

Discussion

An ESHRE consensus meeting report stated that the essential aim of IVF/ICSI is the birth of one single healthy child, with a twin pregnancy being regarded as a complication (Land and Evers, 2003). The collection of a mature oocyte from a naturally selected follicle, followed by IVF, has received increasing attention as an alternative to conventional IVF treatment. The benefits of natural IVF compared with conventional IVF include reduced cost (fewer drugs), reduced health risks (no hyperstimulation syndrome and multiple pregnancies) and increased patient acceptability (no blood tests). The financial benefits of natural IVF as compared with conventional IVF have been reported previously (Daya et al., 1995).

Although this protocol does require the patient to take some medication, it is greatly reduced as compared with normal stimulation protocols in conventional IVF, even including mild stimulation. The oocyte retrieval procedure with only one follicle is extremely quick and may be performed without anaesthesia or sedation. Since only one oocyte and, therefore, one embryo is obtained, there is no risk for multiple pregnancy.

The International Society of Mild Approaches in Assisted Reproduction (ISMAAR) is an organization recently founded to promote a more physiological, less drug-oriented, lower risk, less expensive and more patient-friendly approach to assisted reproduction technology and this includes natural IVF, especially with more recent research suggesting an advantage in embryo implantation and a decrease in oocyte abnormalities (Nargund and Frydman, 2007).

Gonadal agenesis with a 46,XX chromosome complement is an extremely rare disorder affecting females with only 11 cases reported in the literature. Out of those reported cases; only eight of them reached the age of puberty and exhibited primary amenorrhoea, lack of secondary sexual development and hypergonadotrophic hypogonadism. Variable forms of Müllerian anomalies were observed in all of these patients.

As far as is known, this report describes the first case of a female patient with ovarian agenesis and normal Müllerian organs who was able to carry a pregnancy and have a live birth using oocyte donation. In addition, it is the second published report of a clinical pregnancy and live birth utilizing natural IVF in an oocyte donor cycle, the preceding publication reported two ongoing pregnancies in five recipients with natural-cycle oocyte donation from five donors using a GnRH antagonist (Meldrum et al., 1994).

In summary, this report indicates that natural IVF is a viable option for infertile women who seek treatment through oocyte donation with the advantage of less medication, less risk of multiple gestations and significant financial advantage for the concerned couple.

References


Dede M, Gezginc K, Ulubay M et al. 2008 A rare case of rudimentary uterus with absence of both ovaries and 46,XX normal karyotype without mosaicism. Taiwan Journal of Obstetrics and Gynecology 47, 84–86.


Mutchinick OM, Morales JJ, Zentenco IC et al. 2005 A rare case of gonadal agenesis with paramesonephric derivatives in a patient with a normal female karyotype. Fertility and Sterility 83, 201–204.


Declaration: The authors report no financial or commercial conflicts of interest.

Received 1 September 2008; refereed 7 October 2008; accepted 20 February 2009.
Appendix 8


Contribution by SJ Phillips
Manuscript writing and editing

Citation Metrics
Google Scholar: 0
Scopus: 0

In the following paper a grammatical error has been noted; on page 111, the second and third paragraphs of the Discussion should read:

Primary ovarian insufficiency affects 1-5% of women. Eighty percent of POI remains idiopathic after a well-established workup. For a long time, patients with POI were considered irreversibly infertile. Many therapies were used (clomiphene citrate, gonadotropins, estrogens, GnRH, combinations of these and egg donation) but only 6.3% of those patients became pregnant.

Dehydroepiandrosterone is an endogenous steroid produced by both theca cells in the ovary and from the zona reticularis of the adrenal cortex. It is the main source of androgens in women through its conversion to testosterone. It is then transformed into estradiol under the action of the aromatase. Finally, DHEA is a prohormone essential to ovarian steroidogenesis.
Ovarian rejuvenation with dehydroepiandrosterenedione prior to a modified natural in vitro fertilization cycle: A new hope in premature ovarian insufficiency

Pierre Lehmann¹², Eric Himaya¹², Simon Phillips², Isaac-Jacques Kadoch¹²

¹Department of Obstetrics and Gynecology, University of Montreal, Montreal, QC H3C 3J7, ²Clinique OVO, 8000 Blvd. Décarie, Suite 600, Montréal, QC H4P 2S4, Canada

Address for Correspondence: Dr. Pierre Lehmann, E-mail: p.lehmann83@gmail.com

ABSTRACT

Patients with primary ovarian insufficiency (POI) are classically oriented to egg donation due to the lack of currently available efficient treatments. In response to this shortcoming, modified natural in vitro fertilization cycle (mnIVF) combined with dehydroepiandrosterenedione (DHEA) neoadjuvant treatment is an original therapeutic tool at our disposal. A 33-year-old patient with idiopathic POI was prescribed DHEA neoadjuvant treatment. She rapidly recovered her menstrual cycle and after 3 months of DHEA, basal follicle-stimulating hormone has decreased drastically. Then, a mnIVF was performed. The patient got pregnant and gave birth to a healthy girl. She became pregnant a second time under DHEA only. Coupled with DHEA neoadjuvant treatment, a mnIVF could be a useful therapeutic tool for extremely poor prognosis patients with POI.

Key Words: Androgen, dehydroepiandrosterenedione, modified natural in vitro fertilization, ovarian rejuvenation, primary ovarian insufficiency

INTRODUCTION

With the lack of evidence-based medicine, primary ovarian insufficiency (POI) patients are often rapidly oriented toward egg donation. The hypergonadotropic hypogonadism exposes patients to chronically elevated levels of follicle-stimulating hormone (FSH) that down regulate FSH receptors on the surface of granulosa cells. The follicle is therefore desensitized to FSH. By lowering endogenous FSH, it would be possible to restore ovulation and possibly to obtain a spontaneous pregnancy. There are many options to lower endogenous FSH.

Our team has developed and implemented a multidisciplinary approach centered on patient care. Modified-natural in vitro
fertilization (mnIVF) cycle combined with adjuvant dehydroepiandrosterenedione (DHEA) administration is an original therapeutic option.

**CASE REPORT**

A 33-year-old woman presented to the clinic with a history of primary infertility for 18 months. She gradually had shorter cycles in the past year, going from 28 to 23 days cycles. For the past 5 months, she had a secondary amenorrhea.

Of interest, her family history revealed that her mother had a premature menopause at the age of 39. The patient's FSH was 67 IU/L and 60.8 IU/L on two samples at a 1-month interval. Her anti-Müllerian hormone (AMH) was measured at 0.2 ng/mL. A vaginal ultrasound (GE Voluson E8) showed a surprising total antral follicle count of 13 between 2 mm and 9 mm (10 follicles on the right: 3, 3, 4, 5, 5, 7, 7, 8, 8 mm, and 3 on the left: 3, 6, 7 mm). The POI workup including a karyotype, an FMR1 mutation screen and an autoimmune investigation (anti-21-hydroxylase and anti-thyroid antibodies) was negative. She was therefore diagnosed with idiopathic POI.

After beginning a treatment of DHEA 75 mg daily, the patient rapidly re-established a regular menstrual cycle. Three months after the treatment, the basal FSH dropped from 60.8 IU/L to 10.85 IU/L. However, the AMH remained low at 0.16 ng/mL. Then, mnIVF cycles were started using the following usual protocol in our center. Ultrasound monitoring is started on cycle day 9 for patients with regular cycles between 28 and 30 days. Shorter cycles are seen sooner. Vaginal ultrasound is used in order to closely examine the endometrium and the ovaries. Once follicular size reaches 15 mm with an endometrial thickness of at least 6 mm, a gonadotropin-releasing hormone (GnRH) antagonist (Ganirelix, Orgalutran; Merck Canada) is introduced at a daily dose of 0.25 mg. We combine the GnRH antagonist with 150 IU of purified urinary human menopausal gonadotropin (hMG) daily (hMG, Repronex; Ferring Canada) until the leading follicle reaches 18 mm. Indomethacin at a dose of 50 mg orally 3 times a day (Indocin, Merck Frosst) is also added until the day after human chorionic gonadotropin (hCG) administration to reduce the risk of premature ovulation. We then administer 5000 IU subcutaneous of hCG (Ferring Canada) to achieve final follicular maturation.

Egg retrieval was performed 34 h after hCG and a mature oocyte was collected. A good morphological embryo was transferred on day 2 after assisted-hatching was performed. Luteal phase support consisted of vaginal progesterone (endometrin 200 mg by day, Ferring Canada) until the 10th gestational week. The patient gave birth to a healthy girl after a pregnancy without incident.

One year later, wishing another pregnancy, she became pregnant a second time under DHEA only, before even starting mnIVF.

As usual, an informed written patient consent was obtained to present her case.

**DISCUSSION**

The first pregnancies after IVF were obtained in the late 1970s with oocytes retrieved from natural cycles. In the 1980s, stimulated cycles grew in popularity with more oocytes retrieved and more flexible protocols. At present, interest has grown toward mnIVF that offer a reduction in costs, in ovarian hyperstimulation syndrome, in multiple pregnancies and their complications.

Primary ovarian insufficiency affects 1-5% of women. Eighty percent of POI remains idiopathic after a well-established workup. For a long time, patients with POI were considered irreversibly infertile. Many therapies were used (clomiphene citrate, gonadotropins, estrogens, GnRH, combinations of these and egg donation) but only 6.3% of those patients become pregnant.

Dehydroepiandrosterenedione is an endogenous steroid produced by both theca cells from the ovarian zona reticula and the adrenal cortex. It is the main source of androgens in women through its conversion to testosterone. It’s then transformed into estradiol under the action of the aromatase. Finally, DHEA is a prohormone essential to ovarian follicle steroidogenesis.

In 2000, the benefits of DHEA in assisted reproduction for the treatment of poor responders to ovarian stimulation were first presented by Casson et al. This was later confirmed by studies from Barad and Gleicher. In a prospective randomized controlled study in 2010. Wiser et al. showed higher pregnancy rates in poor responders after a 6 weeks 75 mg daily DHEA treatment prior to ovarian stimulation (23.1% vs. 4%, P = 0.05).

Over the last decade, DHEA has gained the interest of numerous specialists for the treatment of POI, premature ovarian aging or diminished ovarian reserve sufferers wishing to achieve pregnancy. Only, few cases have been reported concerning the interest of DHEA in POI patients. Mamas and Mamas demonstrated that the use of DHEA lowers endogenous FSH. A 37-year-old woman with a baseline FSH of 102 mIU/mL had a spontaneous...
pregnancy after a 3 month of treatment with 50 mg daily of DHEA. Her FSH dropped to 18.9 mIU/mL. Other similar cases were reported from the same team.\(^\text{[7]}\) This opens the door for a therapeutic use of DHEA for POI patients.

When compared to other androgen preparations (e.g. testosterone), DHEA gives more physiological serum androgen levels. This avoids follicular arrest in the antral stage that usually follows an androgen excess. However, we have to keep in mind that POI patients have 5-10% chance of spontaneous pregnancy.\(^\text{[2]}\)

It is important to cease DHEA administration when pregnancy is confirmed since animal studies have shown a 100% miscarriage rate in rats.\(^\text{[8]}\) However, no side-effect or congenital anomaly has been reported in the previously mentioned human studies.\(^\text{[5]}\)

For patients with POI for whom egg donation remains the main treatment, an adjuvant DHEA treatment coupled with a mIVF cycle can represent an original alternative. Further, randomized controlled studies are needed to assess this practice.\(^\text{[9]}\)

**ACKNOWLEDGMENTS**

The authors would like to thank C. Le Saint (Ovo Laboratory) and S. Brugerie (Ovo Research and Development).

**REFERENCES**

Appendix 9


Contribution by SJ Phillips

Data collection
Data analysis
Manuscript writing and editing

Citation Metrics

Google Scholar: 11
Scopus: 7
Web of Science: 5
Outcomes of 1503 cycles of modified natural cycle in vitro fertilization: a single-institution experience

Talya Shaulov1 · Maria P Vélez1,2 · Karen Buzaglo1,2 · Simon J Phillips2 · Isaac Jacques Kadoch1,2

Abstract

Purpose A retrospective cohort study was conducted in a single academic center to determine if modified natural cycle in vitro fertilization (mnIVF) is an acceptable treatment for the infertile couple.

Methods Cycles performed between July 2005 and December 2011 were included. In our center’s mnIVF protocol, a GnRH antagonist, gonadotrophin, as well as Indocid are given on a daily basis from detection of a dominant follicle until ovulation induction. The primary outcomes were clinical pregnancy rates (CPR) per cycle started and per embryo transfer (ET). Outcomes were stratified by female patient age (≤35 years and ≥36 years). They were further stratified in each age group by ovarian response status according to the 2011 Bologna criteria.

Results A total of 1503 cycles of mnIVF, performed in 782 patients, were analyzed. CPRs were 13.7 % per started cycle and 32.5 % per ET. Stratification by ovarian response status (normal or poor) in each age group showed similar CPRs in patients ≤35 years (p=0.373), and divergent CPRs per ET in patients ≥36 years old (26.26 vs 6.25 %).

Conclusion MnIVF is an acceptable treatment option for patients considering IVF, particularly for women ≤35 years old and for women ≥36 years old with normal ovarian response.

Keywords Assisted reproduction · Modified natural IVF · Clinical pregnancy rates

Introduction

Conventional stimulated IVF involves various different ovarian stimulation protocols to increase the yield of retrieved oocytes to then increase the chance of fertilized embryos and ultimately to increase the chance of pregnancy. This stimulated form of IVF carries with it a physical and psychological burden for the patient, high cost, a risk of ovarian hyperstimulation syndrome (OHSS), and a risk of multiple pregnancies when more than one embryo is transferred at once. Although the increasing use of antagonist cycles in IVF with a GnRH agonist trigger and a freeze-all strategy has lowered the risk of OHSS, reports in the literature of severe OHSS in these cases are still appearing [1–3]. Also, repeated stimulated IVF cycles are not usually performed in consecutive menstrual cycles in order to allow time for ovaries to recover. For these non-negligible reasons, natural cycle IVF has regained popularity in recent years. Despite the ideal advantages it offers, natural cycle IVF is also associated with high risk of cancelation, which varies in the literature from 15 to 71 % [4]. As this type of IVF revolves around a single follicle, there are several reasons for which the cycle can be canceled: abnormal follicular maturation, premature ovulation of the single follicle, failed oocyte retrieval or empty follicle at retrieval, as well as failed fertilization. However, in contrast to conventional stimulated IVF, repeated cycles can be performed in consecutive months.
Several variations have been put on the spectrum of natural IVF, as described in the literature. These range from a purely natural cycle with no additional medication involved, to a modified natural cycle with the addition of certain agents to prevent premature ovulation and to maintain follicular growth, to mild or minimal stimulation IVF where there is some form of ovarian stimulation with clomiphene citrate or letrozole.

Modified natural IVF (mnIVF), which is used in our center, follows the course of the natural female menstrual cycle. The addition of a GnRH antagonist will help in preventing premature ovulation, and the addition of a gonadotropin will aid in maintaining estradiol concentrations and avoid dominant follicle atresia induced by the GnRH antagonist [5]. Indomethacin has also proved to be beneficial in mnIVF by decreasing premature ovulation rates, and this is when it is initiated prior to the LH surge [6–8]. It does so probably by delaying or preventing spontaneous ovulations through cyclooxygenase blockade, which plays an important role in follicle rupture. Our fertility clinic is a leading center in North America performing modified natural IVF [9], and this technique represents more than 25% of the IVF activity in this center [10].

It is still vague in the literature which patients should be selected for mnIVF. Evidently, only those with regular cycles should be considered since mnIVF depends on the natural dominant follicle. The indication for mnIVF does not seem to affect efficacy of the treatment [11]. Several studies show that natural IVF yields favorable results in poor responders [12–15]. A randomized controlled trial by Morgia et al. [16] comparing purely natural IVF with controlled ovarian stimulation in poor responders demonstrates that natural cycle IVF is a suitable option especially in the younger patients. In 2011, the Bologna criteria for poor ovarian response were established [17] to make it possible to compare different studies on this subject. Following this, a retrospective cohort trial [18] comparing poor responders, defined by the Bologna criteria, to normal responders in natural cycle IVF showed contradictory results to the studies previously stated. Also, a recent study looking at outcomes of mnIVF in poor responders, with a protocol very similar to the one used in our center, showed that this treatment yields poor outcomes in this patient population [19].

The objective of the present study is to describe the outcomes of a large number of cycles of mnIVF cycles performed in our center and then analyze them according to patients’ age and ovarian response status as per Bologna criteria.

Materials and methods

Study design

A single-center retrospective cohort study was performed using all mnIVF cycles performed between July 2005 and December 2011, according to the current mnIVF protocol used in our center. Cycles that diverged from this protocol were excluded.

The decision to undertake mnIVF is a shared decision between the healthcare professional and the patient. Some patients had undergone stimulated cycles beforehand, and some patients were undergoing mnIVF as their first IVF treatment. In our practice, mnIVF is strongly recommended as a first-line treatment for young patients with poor ovarian reserve testing and with an indication for IVF [9, 20].

The primary end points studied were clinical pregnancy rates per cycle started and per embryo transfer. A clinical pregnancy was defined as the visualization of a positive fetal heartbeat on ultrasound. Secondary endpoints were cancellation rates, successful oocyte retrieval rates, fertilization rates, and embryo transfer (ET) rates.

Outcomes were stratified by female patients’ age (≤35 years and ≥36 years) and were further stratified in each age group by poor ovarian response according to the 2011 Bologna criteria (Ferraretti et al., 2011). Women are classified as poor responders when at least two of three criteria are met: (i) advanced maternal age (≥40 years) or any risk factor for poor ovarian response, (ii) a previous poor ovarian response (≤3 oocytes with a conventional stimulation protocol), (iii) an abnormal ovarian reserve test (i.e., antral follicle count <5–7 follicles or AMH <0.5–1.1 ng/ml). Patients who had undergone previous stimulated IVF cycles were evaluated based on all three criteria, and patients not having done so were evaluated based only on the first and third criteria.

Details of the cycle protocol

In our mnIVF protocol, an ultrasound is performed on day 9 of the cycle to detect the presence of a dominant follicle. Monitoring is then continued with ultrasound. Once the dominant follicle reaches 15 mm, a 0.25-mg subcutaneous injection of a GnRH antagonist (Orgalutran®—MSD Canada or Cetrotide®—EMD Serono Canada), a 150-IU subcutaneous injection of a gonadotrophin (Repronex® or Menopur®, Ferring Canada), as well as a 50-mg suppository of Indocid (Merck Canada) three times daily are given on a daily basis until ovulation induction day. Ovulation is induced by a 5000-IU hCG injection when the dominant follicle attains maturity (18–19 mm). Oocyte retrieval is then performed 34 h later under minimal or no sedation according to patient preference, and the embryo is transferred at cleavage stage on day 2. Patients were offered ICSI if male factor contributed to the infertility. Cycles were canceled when an ovarian cyst was detected during follicular surveillance, if the dominant follicle failed to progress normally, or if ovulation happened prior to oocyte retrieval. Luteal phase support was given in the form of intravaginal progesterone.
Statistical analysis

Proportion comparisons were performed by chi-squared test or Fisher’s exact test when indicated. A p value <0.05 was considered to be statistically significant. Statistical analysis was performed using STATA version 10.0 (Stata Corporation, College Station, TX, USA).

This retrospective study was approved by the institutional Research Ethics Committee on 4 January 2012.

Results

Overall outcomes

A total of 1503 cycles of mIVF were included in this analysis, which were performed in 782 patients. Baseline patient characteristics are shown in Table 1. Primary infertility was the case in 494 patients, and the most frequent cause of infertility was male factor. The mean number of days that the antagonist, gonadotrophin, and indocid were given was 2.61 days, with a range of 1 to 8 days. A maximum of 5 cycles was performed per patient; however, the number of cycles performed decreased from 782 to 21 from the first to the fifth rank.

Of the 1503 cycles, 279 were canceled (18.6 %). The two most common causes of cancelation were absent or abnormal follicular recruitment in 94 cycles and premature ovulation in 86 cycles. The rest were due to other causes (e.g., cyst formation) or not specified.

Table 1 Characteristics of patients undergoing up to five cycles of mIVF

<table>
<thead>
<tr>
<th>Number of patients (N)</th>
<th>782</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female patient age, mean (SD)</td>
<td>32.5 (3.2)</td>
</tr>
<tr>
<td>≤35 years old, n (%)</td>
<td>657 (84.0)</td>
</tr>
<tr>
<td>≥36 years old, n (%)</td>
<td>125 (16.0)</td>
</tr>
<tr>
<td>Male patient age, mean (SD)</td>
<td>36.7 (6.0)</td>
</tr>
<tr>
<td>Type of infertility, n (%)</td>
<td>494 (63.4)</td>
</tr>
<tr>
<td>• 1°</td>
<td>286 (36.6)</td>
</tr>
<tr>
<td>• 2°</td>
<td>704 (90)</td>
</tr>
<tr>
<td>Ovarian reserve by Bologna criteria, n (%)</td>
<td>70 (10)</td>
</tr>
<tr>
<td>• Normal</td>
<td>598 (76.5)</td>
</tr>
<tr>
<td>• FSH ≤10 IU/L, n (%)</td>
<td>184 (23.5)</td>
</tr>
<tr>
<td>Cause of infertility, n (%)</td>
<td>71 (8.9)</td>
</tr>
<tr>
<td>• Tubal</td>
<td>272 (34.78)</td>
</tr>
<tr>
<td>• Endometriosis</td>
<td>85 (10.87)</td>
</tr>
<tr>
<td>• Low ovarian reserve</td>
<td>129 (16.5)</td>
</tr>
<tr>
<td>• Unexplained</td>
<td>189 (24.17)</td>
</tr>
</tbody>
</table>

Table 2 presents the overall cycle outcomes, as well as per patient cycle. Overall, oocyte retrieval was attempted in 1224 cycles (81.4 %) and was successful in retrieving an oocyte in 1090 cycles (89.1 %). Successful fertilization was achieved in 729 cycles (66.9 % per successful oocyte retrieval), and embryo transfer was performed in 628 cycles (41.8 %). Single-embryo transfer (SET) was performed in all cases. The clinical pregnancy rate per cycle started, per oocyte retrieval, and per embryo transfer was 13.7, 18.9, and 32.5 %, respectively.

Results by patients’ age

There were 625 patients in the group 35 years or younger who completed 1238 cycles, and there were 125 patients in the group 36 years or older who completed 265 cycles. There was a higher cancelation rate in the group of patients over 35 years old (23.8 vs 17.4 %), but this difference did not reach statistical significance (p=0.12). The rate of successful oocyte retrieval per attempt was equivalent in both groups (89 vs 89.1 %). There was a slightly higher fertilization rate in the older group of patients (65.8 vs 72.2 %), which was not significant. There was an almost equivalent rate of ET per cycle in both groups (41.6 vs 43 %). In the younger group, the clinical pregnancy rates per cycle started, per oocyte retrieval, and per embryo transfer were 14.5, 19.7, and 34.5 %, respectively. These rates, which were all lower in the older group, were 10.2, 15, and 23.5 %, respectively. The differences that were significant between the two age categories were the clinical pregnancy rates per oocyte retrieval and per ET (p=0.02).

Results by Bologna criteria

There were a total of 704 normal responders and 78 poor responders. In each age category (≤35 years old, ≥36 years old), we stratified patients and their respective cycles by poor or normal ovarian response. In the group ≤35 years old (Table 3), 1119 cycles came from patients with normal ovarian response and 119 from patients with low ovarian response. The cancelation rates in normal responders were lower than in poor responders (16.7 vs 24.3 %); however, this difference was not significant (p=0.152). The remainder of patients went on to have oocyte retrieval, and the success rates in both categories were similar (89.4 vs 84.4 %), as were the rates of ETs per started cycle (41.2 vs 45.4 %). In regard to clinical pregnancies, there were 163 pregnancies among normal responders and 16 among poor responders, giving rates of 14.6 and 13.5 %, respectively, per started cycle, and 35 and 29.6 %, respectively, per ET. These differences were not significant.

In the group of patients aged ≥36 years (Table 3), there were 214 cycles from normal responders and 51 cycles from poor responders. The cancelation rate among normal
responders was, once again, not significantly lower than among poor responders (22.9 vs 27.5 %). The two categories had similar successful oocyte retrieval rates (89.1 vs 86.8 %). There was a trend toward a higher ET rate in normal versus poor responders (46.3 vs 31.4 %), which was on the limit of statistical significance ($p = 0.05$). There were 26 clinical pregnancies among normal responders, and only 1 pregnancy among poor responders, resulting in clinical pregnancy rates of 12.2 and 1.96 %, respectively, per started cycle ($p = 0.04$) and 26.3 and 6.25 %, respectively, per ET ($p = 0.06$).

**Discussion**

To our knowledge, this is the largest series of mnIVF cycles and the first from a North American center. This is a representative sample, as consecutive patients were included. A total of 74 cycles were excluded for diversion from the current protocol. We provide the results for all patients combined and then distinguished by indication for IVF, by age, and by ovarian response. Overall, the clinical pregnancy rate per cycle was 13.7 and 32.5 % per ET.

The cancelation rate of 18.6 % in our cohort is comparable to the 19.3 % of another large study of 1048 cycles of mnIVF which had a similar cycle protocol [21] and lower than the 28.9 % cancelation rate described in purely natural IVF cycles [4]. This further highlights the value of the short course of GnRH antagonists, gonadotrophins, and Indocid, which have been proven beneficial in lowering premature ovulation rates [6, 7, 22]. We had a fairly high number of cycles canceled due to abnormal or absent follicular recruitment (6.3 %), and

<table>
<thead>
<tr>
<th>Cycle number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles started</td>
<td>782</td>
<td>436</td>
<td>189</td>
<td>75</td>
<td>21</td>
<td>1503</td>
</tr>
<tr>
<td>Cycles canceled, n (% per cycle)</td>
<td>141 (18.0)</td>
<td>85 (19.5)</td>
<td>36 (19.0)</td>
<td>11 (14.7)</td>
<td>6</td>
<td>279 (18.6)</td>
</tr>
<tr>
<td>1. Premature ovulation</td>
<td>38 (4.8)</td>
<td>26 (6.0)</td>
<td>13 (6.9)</td>
<td>7 (9.4)</td>
<td>2 (9.5)</td>
<td>86 (5.7)</td>
</tr>
<tr>
<td>2. No dominant follicle</td>
<td>52 (6.6)</td>
<td>31 (7.1)</td>
<td>8 (4.2)</td>
<td>3 (4.0)</td>
<td>–</td>
<td>94 (6.3)</td>
</tr>
<tr>
<td>3. Cyst</td>
<td>20 (2.6)</td>
<td>10 (2.3)</td>
<td>6 (3.2)</td>
<td>–</td>
<td>1 (4.8)</td>
<td>37 (2.5)</td>
</tr>
<tr>
<td>4. Other</td>
<td>31 (4.0)</td>
<td>18 (4.1)</td>
<td>9 (4.7)</td>
<td>1 (1.3)</td>
<td>3 (14.3)</td>
<td>62 (4.1)</td>
</tr>
<tr>
<td>ORs (oocyte retrieval) performed, n (%/cycle)</td>
<td>641 (90.6)</td>
<td>351 (80.5)</td>
<td>153 (81.0)</td>
<td>64 (85.3)</td>
<td>15 (71.4)</td>
<td>1224 (81.4)</td>
</tr>
<tr>
<td>Successful OR, n (% per attempt)</td>
<td>581 (74.3)</td>
<td>305 (86.9)</td>
<td>132 (86.3)</td>
<td>57 (89.1)</td>
<td>15 (100)</td>
<td>1090 (89.1)</td>
</tr>
<tr>
<td>Cycles with fertilization, n (% per successful OR)</td>
<td>390 (67.1)</td>
<td>200 (65.6)</td>
<td>87 (65.9)</td>
<td>41 (71.9)</td>
<td>11 (73.3)</td>
<td>729 (66.9)</td>
</tr>
<tr>
<td>Embryo transfers (ET), n (% per cycle)</td>
<td>334 (42.7)</td>
<td>170 (40)</td>
<td>80 (42.6)</td>
<td>37 (49.3)</td>
<td>7 (33.3)</td>
<td>628 (41.8)</td>
</tr>
<tr>
<td>Clinical pregnancies per cycle, %</td>
<td>15.0</td>
<td>13.5</td>
<td>10.1</td>
<td>10.7</td>
<td>14.3</td>
<td>13.7</td>
</tr>
<tr>
<td>Clinical pregnancies per OR, %</td>
<td>20.1</td>
<td>19.3</td>
<td>14.4</td>
<td>14.0</td>
<td>20.0</td>
<td>18.9</td>
</tr>
<tr>
<td>Clinical pregnancies per ET, %</td>
<td>34.5</td>
<td>34.7</td>
<td>23.5</td>
<td>21.6</td>
<td>42.9</td>
<td>32.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ovarian response</th>
<th>≤35 years old</th>
<th>≥36 years old</th>
<th>p value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles started, n (%)</td>
<td>1119 (90.4)</td>
<td>214 (80.8)</td>
<td>0.152</td>
<td>0.143</td>
</tr>
<tr>
<td>Cycles canceled, n (%)</td>
<td>188 (16.8)</td>
<td>49 (22.9)</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>OR (oocyte retrieval) performed, n (% per cycle)</td>
<td>933 (83.34)</td>
<td>165 (77.1)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Successful OR, n (% per attempt)</td>
<td>834 (89.39)</td>
<td>147 (89.09)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Cycles with fertilization, n (% per successful OR)</td>
<td>541 (64.87)</td>
<td>109 (74.15)</td>
<td>0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Embryo transfers (ET), n (% per cycle)</td>
<td>461 (41.20)</td>
<td>99 (46.26)</td>
<td>0.349</td>
<td>0.054</td>
</tr>
<tr>
<td>Clinical pregnancies, n</td>
<td>163</td>
<td>26</td>
<td>0.404</td>
<td>0.04</td>
</tr>
<tr>
<td>% per cycle started</td>
<td>14.57</td>
<td>12.15</td>
<td>0.014</td>
<td>0.035</td>
</tr>
<tr>
<td>% per successful OR</td>
<td>19.57</td>
<td>17.69</td>
<td>0.373</td>
<td>0.064</td>
</tr>
<tr>
<td>% per ET</td>
<td>35.05</td>
<td>26.26</td>
<td>0.62</td>
<td>0.064</td>
</tr>
</tbody>
</table>
this may be influenced by the inclusion of several patients with irregular periods (i.e., anovulatory cycles).

Our pregnancy rate per started cycle of 13.7% is higher than that of 7.9% described by Pelinck et al. [21] (p<0.001). It is interesting to note that the cycles were performed only in patients aged 18–36 years. Differences in the indication of mnIVF or in the selection of patients for mnIVF in their centers may explain this disparity.

In our study, most patients were 35 years or younger and had a normal ovarian response, leaving few patients and evidently few cycles in the groups ≥36 years old and poor ovarian response. The clinical pregnancy rate in women aged ≤35 years was 14.5% per cycle and 34.5% per embryo transfer. In women aged ≥36 years, these rates were 10.2% per cycle and 23.5% per ET. This observation that outcomes are better in younger patients is not surprising as follicular quality decreases with increasing age, and it confirms those of several other studies [23–25, 15].

We then further stratified the two age groups by ovarian response status according to the 2011 Bologna criteria, as clinically, this population often poses a management quandary. The results of our current analysis cannot easily be compared to most other studies involving poor responders, as the definition of poor responders is inconsistent among these. However, there are two more recent studies which use these latest Bologna criteria to evaluate natural cycle IVF treatment in poor responders [18, 19]. The earlier one by Polyzos et al. [18] concludes that poor responders do not respond well to natural cycle IVF, with ongoing pregnancy rates per cycle under 3% in all age groups in this category. On the contrary, patients classified as normal responders gave significantly better results with a live birth rate per cycle of 8.9% (ongoing pregnancy rates not mentioned). The results of our study differ in that the poor responders ≤35 years old seem to respond equally as well as the normal responders, with respective clinical pregnancy rates of 13.45 and 14.57% (p=0.373). However, we concur that poor responders ≥36 years old do not benefit from this treatment. These latter results we present are also in line with those of Kedem et al. [19], who studied the outcomes of a similar mnIVF protocol in poor-responder women with an average age of 39 years old and show very poor outcomes in this group. Proposed theories to support the advantage of natural IVF over stimulated IVF are that the follicle naturally selected is of better quality and the endometrium is more receptive. In older patients, as follicular quality naturally declines so does the response to any kind of assisted reproductive technique [26], which explains the negligible chance of pregnancy when combined with poor ovarian response.

We note that the selection of patients for natural cycles in our center may create a bias in the results. In other words, given that the majority of patients did not undergo stimulated IVF prior to mnIVF, their eligibility for one of the three Bologna criteria is removed. It is possible that had they undergone a stimulated IVF cycle, a certain number may have responded poorly, increasing the number of poor responders in this study, and shifting the results either more or less favorably toward the poor responders.

A weakness of our study is that due to its retrospective design, patients who decided not to undergo further treatments despite unsuccessful attempts were lost to follow-up. These dropouts may have been due to patients changing fertility centers, reaching spontaneous pregnancy, or proceeding to stimulated IVF.

We consider that including male factor infertility had no impact on our results. A previous report evaluating results of mnIVF-ICSI cycles using ejaculate or surgically retrieved sperm for male factor infertility and performed in women under 37 years of age showed similar clinical pregnancy rates per ET to those reported in our study (31.0% and 35.1% with ejaculated and surgically retrieved sperm, respectively) [27].

A strength of this large series is that all cycles were performed according to one protocol which allows conclusions to be drawn about modified natural IVF specifically—an aspect that is especially relevant when counseling patients before treatment. Studies which combine both natural and minimal stimulation IVF cannot be interpreted in the same way in this regard [23, 25].

The attractiveness of modified natural IVF evidently comes from its easy accessibility, minimal risk of ovarian hyperstimulation, low rate of multiple pregnancies, and low cost. Also, these cycles have the advantage of being able to perform the oocyte retrieval with minimal or no sedation as it is quite a short procedure requiring only one needle passage through the vagina. However, the challenge remains selecting the right patient population. Our study shows that mnIVF is a reasonable treatment option for patients considering IVF, specifically for those ≤35 years old with normal or poor ovarian response, possibly for those ≥36 years old with normal ovarian response, and may even be considered as a first approach in these patients. As single-embryo transfer gains popularity in IVF, mnIVF may be a less aggressive but effective approach to attain this embryo in these good prognosis patients. However, in poor responders ≥36 years old, mnIVF is not justified, and this confirms findings of recent studies. Randomized trials are necessary to determine the optimal conditions for mnIVF, such as the timing of ovulation induction or endometrial thickness at induction, in order to maximize chance of pregnancy and to minimize chance of cancellation. Also,
further analyses by indication are necessary as current evidence on this matter is not uniform.

Acknowledgments We would like to acknowledge the work of the research staff for their implication in this study: Cecile LeSaint, Shirley Brugerie, Marie-Paule Lachambre, and Cynthia Levesque.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. For this type of study, formal consent is not required.

Funding and financial disclosure None on behalf of all authors.

Conflict of interest The authors declare that they have no conflict of interest.

References

Appendix 10


**Contribution by SJ Phillips**

- Data collection
- Manuscript writing and editing

**Citation Metrics**

- Google Scholar: 21
- Scopus: 18
- Web of Science: 12
CASE REPORT

Ongoing twin pregnancy after ICSI of PESA-retrieved spermatozoa into in-vitro matured oocytes

Ahmad Kamal Abdul-Jalil1,3, Tim J.Child1, Simon Phillips1, Nicola Dean1, Serge Carrier2, Seang Lin Tan1

1McGill Reproductive Center, Department of Obstetrics and Gynecology and 2Department of Surgery, McGill University, Montreal, Quebec, Canada
3To whom correspondence should be addressed at: McGill Reproductive Center, Royal Victoria Hospital F6.58, 687 Pine Avenue West, Montreal, Quebec, H3A 1A1, Canada. E-mail: ahmad.kamal@muhc.mcgill.ca

The recovery of immature oocytes from unstimulated ovaries followed by in-vitro maturation (IVM) is an attractive alternative to conventional IVF in the treatment of female infertility. Similarly, surgical recovery of spermatozoa from the epididymis by percutaneous sperm aspiration (PESA) has simplified the retrieval of the male gamete in treatment of men with obstructive azoospermia. We report the first ongoing clinical twin pregnancy resulting from intracytoplasmic sperm injection (ICSI) of spermatozoa retrieved by PESA into IVM oocytes. In the treatment of a 24-year old woman, 12 immature oocytes were retrieved. Six oocytes matured (maturation rate 50%) after 24-hour incubation and were inseminated by ICSI. Four oocytes had two pronuclei (fertilization rate 67%) and 3 good quality embryos were transferred. A viable twin pregnancy was confirmed by ultrasound scan. This report illustrates the use of a combination of less invasive assisted reproductive techniques in overcoming barriers to infertility.

Key words: In-vitro maturation/human oocytes/polycystic ovaries/percutaneous sperm aspiration

Introduction

In in-vitro maturation (IVM) treatment cycles, immature oocytes retrieved from unstimulated ovaries are cultured overnight. Subsequently, matured oocytes are fertilized (Trounson et al., 1994; Barnes et al., 1995). IVM treatment has several advantages including lower treatment cost, reduced health risks and increased patient convenience compared with conventional IVF treatment. Recent reports of live births following IVM treatment of women during unstimulated cycles (Cha et al., 2000, Chian et al., 2000a) have emphasised the significant potential of IVM treatment, especially for women with polycystic ovaries. Similarly, the treatment of male infertility with the use of intracytoplasmic sperm injection (ICSI) has benefited many couples (Palermo et al., 1992). Both ejaculated spermatozoa and those retrieved from the testis and epididymis have been utilized in insemination of oocytes using ICSI (Temple-Smith et al., 1985; Craft and Shrivastav, 1994).

To the best of our knowledge, this is the first report of an ongoing clinical pregnancy resulting from ICSI of spermatozoa retrieved by percutaneous sperm aspiration (PESA), into IVM oocytes.

Case report

A 24-year old woman and her 30-year old husband presented to our clinic with a 3-year history of primary infertility. She had regular menstrual cycles and normal, early follicular and mid-luteal phase serum hormone concentrations. A transvaginal ultrasound examination performed during the early follicular phase diagnosed bilateral polycystic ovaries (Adams et al., 1985).

Her partner had normal sized testes and had previously been treated in another unit with surgical excision of a varicocele that was believed to be the cause of his severe oligozoospermia. Unfortunately, no spermatozoa were found on repeated post-operative semen analysis. His serum concentrations of FSH, LH, testosterone and prolactin were normal. The cause of his azoospermia was considered to be obstructive. The couple had no history of assisted reproduction treatment.

Treatment options including surgical sperm retrieval combined with either IVF or IVM, or donor insemination were discussed with the couple. They opted for IVM treatment with PESA.

On day 3 of a spontaneous menstrual cycle the woman
underwent a baseline transvaginal ultrasound scan to exclude ovarian cysts and to estimate the number of retrievable oocytes. A total of 35 ovarian follicles between 4–6 mm diameter were counted. A second ultrasound scan was performed on day 7 to exclude the development of a dominant follicle and to measure the endometrial thickness. A total of 10 000 IU HCG (Profasi; Serono, Oakville, Ontario, Canada) was administered 36 hours before oocyte retrieval (Chian et al., 2000a). On cycle day 9, transvaginal ultrasound-guided oocyte collection was performed using a specially designed 17G single-lumen aspiration needle (K-OPS-1235-Wood, Cook, Queensland, Australia) with a reduced aspiration pressure of 7.5 kPa. Aspiration of all small follicles was performed without flushing. Oocyte retrieval was performed under i.v. sedation (2 mg Midazolam and 175 μg Fentanyl) and a paracervical block with 10 ml of 1% lidocaine. Oocytes were collected in 10 ml culture tubes containing 2 ml warm 0.9% saline with 2 IU/ml heparin (Baxter, Toronto, Ontario, Canada). Cumulus-oocyte complex (COC) were identified, washed and incubated in an organ tissue culture dish (60×15 mm; Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA) containing 1 ml of maturation medium. The IVM medium consisted of TCM-199 (Sigma Chemical Co., St Louis, MO, USA) supplemented with 75 mIU/ml FSH and LH (Humegon; Organon, Scarborough, Ontario, Canada), 25 mol/l pyruvic acid (Sigma Chemical Co.) and 20% heat-inactivated maternal serum. The oocytes were incubated in an incubator at 37°C in an atmosphere of 5% carbon dioxide and 95% air with high humidity.

Twenty-four hours post-collection, the oocytes were denuded with hyaluronidase (Scandinavia IVF Science, Gothenburg, Sweden) and mechanical pipetting. Mature (metaphase II) oocytes were identified by the presence of the first polar body. Immature oocytes were further cultured in IVM medium, and examined prior to ICSI and 48 hours post-collection.

On the day of ICSI, the husband produced two ejaculated semen samples, but no spermatozoa were found in either specimen. A percutaneous epididymal sperm aspiration (PESA) procedure was performed. Epididymal fluid was collected with a syringe connected to a 21G butterfly needle, which was inserted directly through the skin and the epididymal tubule. The aspirated fluid from the right testis was examined under the microscope. Motile spermatozoa were observed, isolated and then washed (200 g for 12 min) and resuspended in IVF-20 medium (Scandinavia IVF Science). The spermatozoa were incubated (37°C, 5% carbon dioxide) until use. Mature oocytes were inseminated by ICSI and cultured in organ tissue culture dish (60×15 mm; Falcon). After the ICSI procedure, surplus spermatozoa were cryopreserved.

For endometrial preparation, the patient was given 6 mg of oestradiol valerate (Estrace; Roberts Pharmaceutical, Mississauga, Ontario, Canada) in divided doses starting on the day of oocyte retrieval. Endometrial thickness on the day of oocyte collection was 7.5 mm. Luteal support was provided in the form of 400 mg of progesterone twice a day for 16 days starting on the day of ICSI until 12 weeks gestation.

Twelve immature oocytes were retrieved at oocyte collection. Six mature oocytes (50% maturity rate) were obtained after 24 h culture in maturation medium and were inseminated by ICSI. Upon examination of the injected oocytes 18 h later, four had two pronuclei (fertilization rate 67%). No additional mature oocytes were obtained after 48 h culture. Three good quality embryos (one embryo 4 cells, grade 1; 2 embryos 4 cells, grade 2) were transferred 48 hours after ICSI. The serum β-HCG concentration 14 days after transfer was 1824 IU/ml. Two weeks later, a clinical twin pregnancy was confirmed with two fetal hearts seen at transvaginal ultrasonography. A subsequent scan at 12 weeks gestation confirmed viability of both fetuses.

Discussion
The collection of immature oocytes from unstimulated ovaries, followed by in-vitro oocyte maturation and subsequent insemination, has received increasing attention as an alternative to conventional IVF treatment (Cha and Chian, 1998; Chian et al., 2000a). The benefits of IVM compared with IVF include reduced cost [less drugs and monitoring], reduced health risks (no gonadotrophin associated ovarian hyperstimulation syndrome (OHSS) or putative future ovarian cancer risk], and increased patient acceptability (reduced blood test and ultrasonographic monitoring and no daily injections). IVM is particularly useful for patients with polycystic ovaries, who have an increased risk of developing OHSS (MacDougall et al. 1993) and who produce a significant number of immature oocytes following ovarian stimulation (Dor et al., 1990) resulting in lower fertilization rates (MacDougall et al., 1993; Kodama et al., 1995, Aboulghar et al., 1997).

Though Cha et al. (1991) were first to describe the successful use of IVM in a donor oocyte programme (Cha et al., 1991), Trounson and colleagues (1994) placed IVM in the clinical realm when they first reported a pregnancy using oocytes retrieved by transvaginal ultrasound-guided follicle aspiration from a patient with polycystic ovaries (Trounson et al., 1994). Since then, there have been a number of reports of the successful use of IVM, but pregnancy rates and live births associated with IVM have remained relatively low (Barnes et al., 1995; Cha and Chain, 1998). Recently, our group showed that the maturation rate can be improved by the addition of HCG priming before immature oocyte retrieval in women with polycystic ovaries (Chian et al., 1999, 2000a). High maturation, fertilization and cleavage rates were obtained, resulting in a clinical pregnancy rate of 39% per cycle commenced. Several live births following IVM treatment of women during unstimulated cycles have been reported (Cha et al., 2000, Chian et al., 2000a).

It was appropriate that the patient selected IVM as her treatment option since she had a large number of follicles in both ovaries. Her prognosis during the treatment cycle was excellent as she did not develop a dominant follicle (Cobo et al., 1999) by the time of HCG administration and she had a good endometrial thickness of 7.5 mm on the day of oocyte collection (unpublished observation). The thickness of the endometrial lining was achieved by priming with exogenous oestradiol to assist in the proper synchronization of embryo and endometrium development.
The introduction of ICSI (Palermo et al., 1992) has revolutionized the treatment of male infertility. Furthermore, modern sperm recovery techniques have made it possible to help men with obstructive or non-obstructive azoospermia to achieve fatherhood. For men with obstructive azoospermia, high sperm recovery rates from the epididymis have been obtained using microsurgical epididymal sperm aspiration (MESA) (Temple-Smith et al., 1985; Silber et al., 1994) or PESA (Craef and Shrivastav, 1994; Shrivastav et al., 1994).

Before the introduction of ICSI in IVM treatment, fertilization and pregnancy were reported using standard insemination of in-vitro matured oocytes with ejaculated spermatozoa (Cha et al., 1991, Trounson et al., 1994). In IVM treatment, ICSI may be beneficial because of zona changes due to longer in-vitro culture prior to insemination (Nagy et al., 1996; Hwang et al., 2000). ICSI may not, however, always be necessary if ejaculated semen shows normal characteristics and there is a large number of IVM oocytes (Chian et al., 2000b).

The fertilization rate obtained in this case was comparable with those achieved by ICSI of mature oocytes retrieved after ovarian stimulation for conventional IVF, using ejaculated spermatozoa (Tournaye et al., 1995; Nagy et al., 1996). This is consistent with the observations of Ghazzawi et al. (1998) who reported, in a prospective study, that there were no significant differences in fertilization and pregnancy rates following ICSI using spermatozoa from ejaculates, epididymis or testis (Ghazzawi et al., 1998).

Although pregnancies and live births from IVM oocytes have been reported, there has been no report of ongoing clinical pregnancies of IVM-matured oocytes from unstimulated cycles using PESA-retrieved spermatozoa. This report highlights the use of two advanced assisted reproduction techniques to overcome infertility problems faced by the couple, which are relatively less invasive compared with conventional IVF or MESA.

The number of births from IVM is still relatively few. However, the technique has significant potential in the treatment of infertility for women with polycystic ovaries and, with further advances in culture techniques, its use will hopefully be extended to women with normal ovaries.

Note added in proof
The patient had a safe Caesarian-section delivery of healthy twin girls (birthweights of 2200 and 2150 g) at 36 weeks gestation.

References

Received on January 26, 2001; accepted on May 4, 2001
Appendix 11


Contribution by SJ Phillips
   Concept
   Data collection
   Data analysis
   Manuscript writing and editing

Citation Metrics
   Google Scholar: 190
   Scopus: 137
   Web of Science: 115
A Comparison of In Vitro Maturation and In Vitro Fertilization for Women With Polycystic Ovaries

Tim J. Child, MD, MRCOG, Simon J. Phillips, BSc, Ahmad Kamal Abdul-Jalil, MS, Bulent Gulekli, MD, and Seang Lin Tan, MD, FRCOG

OBJECTIVE: To establish the relative success of treatment by unstimulated in vitro maturation (IVM) of oocytes or stimulated in vitro fertilization (IVF) in women with polycystic ovaries undergoing assisted conception treatment.

METHODS: The case-control study included 107 IVM and 107 IVF cycles matched for age and cause of infertility. In vitro maturation patients underwent transvaginal recovery of immature oocytes during an unstimulated cycle, in vitro oocyte maturation, and fertilization. Those in the IVF group underwent ovarian stimulation after pituitary suppression. Embryos were transferred in the same cycle in both groups. Main outcome measures included numbers of mature oocytes and embryos produced, and rates of implantation, pregnancy, live birth, and complications.

RESULTS: In the IVM group after in vitro culture, 7.8 mature oocytes and 6.1 embryos were obtained per retrieval. With IVF, 12.0 mature oocytes (P < .01) and 9.3 embryos (P < .01) were obtained. The IVM pregnancy and live birth rates per retrieval were 26.2% and 15.9% compared with 38.3% and 26.2% for IVF (nonsignificant). The implantation rate of IVF-derived embryos was higher (17.1% versus 9.5%) than that for IVM (P < .01). There were 12 cases (11.2%) of moderate or severe ovarian hyperstimulation syndrome in IVF patients, compared with none in the IVM group (P < .01).

CONCLUSION: Our results suggest that for women with polycystic ovaries who require assisted conception, IVM is a promising alternative to conventional IVF treatment.
MATERIALS AND METHODS

We identified all IVM and IVF treatment cycles performed at the McGill Reproductive Center between February 1998 and November 2000 from our unit database for this case-control study. Cases were defined as IVM cycles in which the patient had a diagnosis of polycystic ovaries. There were a total of 169 IVM cycles performed during the study period, of which 107 were for women with polycystic ovaries. An ovary was considered polycystic if there were ten or more small cysts or follicles visualized that were arranged around the periphery or scattered through an enlarged ovarian stroma on transvaginal ultrasound scan performed during the early follicular phase of the menstrual cycle.

The control for each IVM cycle was defined as the preceding IVF cycle for which the patient was within the same age group (younger than 30, 30–34, 35–39, and older than 39), had the same infertility diagnosis, and had polycystic ovaries on ultrasound examination. All patient files were then manually checked to confirm the polycystic ovaries diagnosis and to assess whether there had been complications such as ovarian hyperstimulation syndrome, pelvic infection, or bleeding during or after the treatment cycle.

During the study period women with polycystic ovaries who required assisted conception were offered, after full explanation, the option of either IVM or IVF treatment. The patient commenced the treatment of her choice after having given written informed consent. The characteristics of women choosing IVM or IVF could have been different. However, by using a case-control study design we were able to match patients for two important baseline variables that may affect outcome—namely, age and diagnosis.

Women with amenorrhea received vaginal progesterone (Prometrium; Schering, Pointe-Claire, Quebec, Canada), 300 mg once daily for 10 days, to induce withdrawal bleeding. All women underwent a baseline ultrasound scan on days 2–4 of menstrual bleeding, whether spontaneous or induced, to ensure that no ovarian cysts were present. No ovarian stimulation was used during the treatment cycle. Transvaginal ultrasound scans were repeated on the day of human chorionic gonadotropin (hCG) administration (2 days before immature oocyte retrieval) to exclude the development of a dominant follicle. All patients received 10,000 IU of hCG (Profasi; Serono, Oakville, Ontario, Canada) subcutaneously 36 hours before oocyte retrieval. We previously demonstrated, in a randomized controlled trial, that hCG priming increases both the percentage and rate of immature oocyte maturation. All follicles had to be less than 10 mm in diameter on the day of hCG to proceed to oocyte retrieval, which was performed between days 9 and 11 of the cycle for women with ovulatory cycles and days 9 and 14 for anovulatory patients. Data suggest that the presence of a dominant follicle at the time of immature oocyte retrieval is deleterious to outcome in IVM.

Transvaginal ultrasound–guided oocyte collection was performed using a specially designed 17-gauge single-lumen aspiration needle (K-OPS-1235-Wood; Cook IVF, Eight Mile Plains, Queensland, Australia) with a reduced aspiration pressure of 7.5 kPa. Aspiration of all small follicles was performed using either spinal anesthesia or intravenous fentanyl and midazolam with or without a paracervical block of 10 mL of 1% lidocaine. Follicular flushing was not performed.

The oocyte IVM technique used in this study has been reported in detail previously. Oocytes were collected in culture tubes containing warm 0.9% saline with 2 IU/mL heparin. The oocytes were evaluated for the presence or absence of a germinal vesicle in the cytoplasm of the oocyte, and the immature oocytes were then transferred into maturation medium for culture. All oocyte-handling procedures were conducted on warm stages and plates at 37°C.

The immature oocytes were incubated in culture dishes containing 1 mL of maturation medium, TC-199 medium supplemented with 20% heat-inactivated maternal serum, 0.25-mmol/L pyruvic acid (Sigma Chemical Co., St. Louis, MO), 50-mg/mL penicillin, 75-mg/mL streptomycin, and 75-mIU/mL FSH and LH (Humegon; Organon, Scarborough, Ontario, Canada) at 37°C in an atmosphere of 5% CO2 and 95% air with high humidity. After culture, the maturity of the oocytes was determined under the microscope at 24 h and 48 h. Mature oocytes were determined by the presence of first polar body extrusion. Oocytes that were mature at the time of checking were denuded of cumulus cells ready for intracytoplasmic sperm injection. A single spermatozoan was injected into each metaphase II oocyte. After intracytoplasmic sperm injection, each oocyte was transferred into a 20-μL droplet of 1.2 medium G (Vitrolife, Göteborg, Sweden). Fertilization was assessed 18 h after intracytoplasmic sperm injection for the appearance of two distinct pronuclei and two polar bodies.

Embryos were transferred on day 2 or 3 after intracytoplasmic sperm injection. Because the oocytes were not matured and inseminated at the same time after maturation in culture, the developmental stages of embryos at the time of embryo transfer often varied. Before transfer, all embryos for each patient were pooled and selected for transfer based on standard embryological criteria such as cleavage stage and morphological quality.

For endometrial preparation, patients received extra-
dil valerate (Estrace; Roberts Pharmaceutical, Mississauga, Ontario, Canada), starting on the day of oocyte retrieval, depending on the endometrial thickness on that day. If the endometrial thickness was less than 6 mm, a 10-mg dose was given, and if it was greater than 6 mm, a 6-mg dose was administered. If the endometrial thickness was less than 7 mm on the day of embryo transfer, the recommendation was cryopreservation of all embryos for replacement in a later cycle. Luteal support was provided by 200 mg of intravaginal progesterone (Prometrium) twice daily starting on the day of intracytoplasmic sperm injection and continued, along with estradiol, until 12 weeks’ gestation.

Women underwent a long gonadotropin-releasing hormone (GnRH) agonist protocol with a follicular phase start. Patients were pretreated with either the oral contraceptive pill for 2 weeks before GnRH agonist or oral progesterone (Norlutate; Pfizer, New York, NY) for 5 days from the first day of menstruation. Ovarian stimulation with urinary or recombinant FSH began after pituitary suppression was confirmed. An appropriate daily FSH dose was selected based on patient age, early follicular phase serum FSH concentration, and any previous response to ovarian stimulation. Human chorionic gonadotropin was administered when there were at least three follicles of at least 18 mm average diameter. Transvaginal ultrasound-guided oocyte collection was performed 36 hours later. Intracytoplasmic sperm injection was performed as required, and cleaving embryos replaced in the uterus 2–4 days later. Patients in both the IVM and the IVF groups were offered the option to cryopreserve any remaining best-quality embryos. Luteal support was provided with progesterone pessaries (Prometrium), 200 mg twice daily, until 12 weeks’ gestation.

For the purposes of this study, patients with ovarian hyperstimulation syndrome were classified into three groups: mild, moderate, and severe. Using this classification, mild ovarian hyperstimulation syndrome includes abdominal distension and discomfort, enlarged ovaries, and/or nausea, vomiting, or diarrhea. Moderate ovarian hyperstimulation syndrome is diagnosed when there is, in addition, ultrasonographic evidence of ascites. Severe ovarian hyperstimulation syndrome is diagnosed when there are clinical ascites, hydrothorax, and/or biochemical or hematological abnormalities.

A pregnancy test was considered positive when a serum hCG taken 16 days after oocyte fertilization had a concentration of greater than 25 IU/L. The implantation rate was calculated by dividing the total number of gestation sacs present on transvaginal ultrasound 4 weeks after oocyte retrieval by the total number of embryos transferred within a treatment group.

Statistical comparisons between categoric data were performed using the $\chi^2$ test. Because none of the oocyte and embryo outcome variables displayed a normal distribution (Kolmogorov-Smirnov test), the non-parametric Mann-Whitney U test was used to analyze differences between unpaired data. All $P$ values quoted are two sided, and values below .05 were taken to indicate statistical significance. Analyses were performed using the SPSS statistical package (SPSS Inc., Chicago, IL).

RESULTS

One hundred seven IVM and 107 IVF cycles were identified as cases and controls, respectively. There were 83 women in the IVM group and 81 in the IVF. There were no differences in mean age, previous number of IVF cycles undertaken, or infertility diagnosis between the treatment groups, indicating satisfactory matching for these variables.

Two cycles in each treatment group failed to reach embryo transfer. Causes were failure to retrieve oocytes in one IVM cycle and maturation failure of the four retrieved immature oocytes in the other. There was no transfer in two IVF cycles because of 1) cross-contamination with Candida albicans from the patient to the embryo culture system and 2) a high estradiol level on the day of hCG administration requiring all embryos to be frozen for transfer in a later cycle because of concern over the risk of ovarian hyperstimulation syndrome.

One thousand one hundred two viable immature oocytes were collected in the IVM group. By 48 hours of culture 835 oocytes had matured to metaphase II (maturation rate 76%). After intracytoplasmic sperm injection of the in vitro–matured oocytes, the fertilization rate was 78% and the cleavage rate was 74%. The fertilization and cleavage rates of IVF oocytes were 78% and 72%, respectively (nonsignificantly different from IVM values).

There were significantly fewer oocytes collected, metaphase II oocytes, fertilized oocytes, and cleaving embryos in the IVM group ($P < .01$) (Table 1). A total of 336 and 284 embryos were transferred to the uterine cavity in the IVM and IVF groups, respectively. Consequently, the mean number transferred in the IVM group (mean ± SD 3.2 ± 0.9, range 1–5) was significantly greater than that in the IVF group (2.7 ± 0.8, range 1–6) ($P < .01$), though the median number transferred was three in both groups.

In vitro maturation cycles appeared less likely to result in pregnancy, clinical pregnancy, and live birth than IVF cycles, though the 95% confidence intervals for these
outcomes just crossed unity, confirming nonsignificance. The implantation rate was significantly lower in the IVM group (9.5%) than in the IVF (17.1%) (\(P < .01\)). There were seven multiple live births in the IVM group (41.2%) and 10 in the IVF (37.0%) (nonsignificant), including one set of triplets in each.

There were 12 cases (11.2%) of moderate or severe ovarian hyperstimulation syndrome in the IVF group as defined using the classification of Golan.\(^{16}\) Seven of these (6.5%) were moderate ovarian hyperstimulation syndrome, and five (4.7%) severe. One patient required admission for 1 night for intravenous hydration, and another underwent transvaginal drainage of ascites, performed as an outpatient procedure. As was expected, there were no cases of ovarian hyperstimulation syndrome in the IVM group (\(P < .01\)).

There were no cases in either group of hemorrhage or pelvic infection requiring antibiotic treatment. One patient in each group was diagnosed with ectopic pregnancy. Both women were treated successfully with systemic methotrexate.

**DISCUSSION**

The results suggest that for women with polycystic ovaries who require assisted conception treatment, IVM is a promising alternative to stimulated IVF. Although the numbers of oocytes and embryos are significantly less, on average, at least five cleaving embryos were available per oocyte retrieval through IVM treatment. These embryos were produced without the need for prolonged, expensive, intrusive, and potentially dangerous gonadotropin ovarian stimulation. The rates of fertilization and embryo cleavage were similar whether or not the oocytes were matured in vitro or in vivo.

We found the implantation rate of embryos derived from in vitro–matured oocytes to be significantly lower than that of IVF embryos. From our data it is not possible to determine whether the lower implantation rate of IVM embryos is a result of reduced oocyte potential, reduced endometrial receptivity, or a combination of both factors.

Explanations for reduced in vitro–matured oocyte developmental potential could include suboptimal culture conditions or defective oocytes due to inadequate cytoplasmic maturation.\(^{17}\) Both nuclear and cytoplasmic maturation, which involve a complex cascade of events, need to be closely integrated to ensure developmental competence. It is possible that, in vitro–matured oocytes, nuclear maturation may be complete, as evidenced by extrusion of the first polar body, whereas cytoplasmic maturation is incomplete. Previous data suggest that, compared with in vivo–matured oocytes, those matured in vitro have a reduced embryo development rate, with increased blockage of cleavage at the zygote stage.\(^{18}\) Our data do not support this finding. However, we did not attempt to culture IVM embryos to the blastocyst stage during the study period, and so cannot comment on developmental blockage beyond day 3 of embryo cul-

| Table 1. Results of 107 Age- and Diagnosis-Matched IVM and IVF Treatment Cycles in Infertile Women With Polycystic Ovaries |
|---|---|---|
| No. of cycles | IVM | IVF | OR (95% CI) |
| Age (y) | 32.8 ± 4.2 | 33.1 ± 4.1 |  |
| Total injected units (ampoules) of follicle-stimulating hormone | 0 | 2355 ± 833 (31.4 ± 11.1)* |  |
| Oocytes collected | 10.3 ± 7.6 | 14.9 ± 6.5* |  |
| Metaphase II stage oocytes | 7.8 ± 4.9 | 12.0 ± 5.4* |  |
| Fertilized 2PN embryos | 6.1 ± 3.8 | 9.3 ± 4.4* |  |
| Cleaving embryos | 5.8 ± 3.7 | 8.6 ± 4.2* |  |
| Embryos transferred (range) | 3.2 ± 0.9 (1–5) | 2.7 ± 0.8 (1–6)* |  |
| Embryos cryopreserved (range) | 0.8 ± 2.3 (0–14) | 1.2 ± 3.0 (0–16) |  |
| Pregnant \(n / \%\) | 28 (26.2) | 41 (38.3) | 0.57 (0.31, 1.06) |
| Implantation rate (\%) | 9.5 | 17.1* | 0.51 (0.31, 0.84) |
| Clinical pregnancy \(n / \%\) | 23 (21.5) | 36 (33.7) | 0.54 (0.28, 1.04) |
| Live birth \(n / \%\) | 17 (15.9) | 28 (26.2) | 0.53 (0.26, 1.10) |
| Multiple live births \(n / \%\) of total live births | 7 (41.2) | 10 (37.0) | 1.26 (0.30, 5.11) |
| Twins | 6 | 9 |  |
| Triplets | 1 | 1 |  |
| Moderate or severe ovarian hyperstimulation syndrome | 0 | 12 (11.2%)* |  |

IVM = in vitro maturation; IVF = in vitro fertilization; OR = odds ratio; CI = confidence interval.

Results are means ± standard deviations unless stated.

*\( P < .01\).
Cytomegalovirus (CMV) infection is the best option, even when the sperm parameters are not impaired. Qualitative changes, including zona hardening, occur in the zona pellucida during oocyte maturation in vitro and may reduce the fertilization rates using conventional IVF. We therefore routinely used intracytoplasmic sperm injection for fertilization of in vitro–matured oocytes.

In our age-matched population the rates of pregnancy and clinical pregnancy per cycle with IVM treatment were 26.2% and 21.5%, respectively, compared with 38.3% and 33.7% for IVF. Similar proportions of cycles with positive serum pregnancy tests in both treatment groups continued on to live birth or an ongoing pregnancy (IVM 61%, IVF 68% [nonsignificant]). Although success rates appear lower, IVM treatment costs less than IVF because less monitoring, both hormonal and ultrasonographic, is needed and no FSH is required. On average, 31.4 ± 11.1 amoules of FSH were administered per IVF cycle. During an IVM cycle only five clinic visits are needed—namely, two ultrasound scans, the oocyte collection, embryo transfer, and serum pregnancy test—resulting in greater treatment simplicity and less disruption to patients’ lives.

The potential risks of ovarian stimulation, particularly for women with polycystic ovaries, are illustrated by the fact that 12 of the IVF cycles (11.2%) resulted in moderate or severe ovarian hyperstimulation syndrome as per the classification of Golan et al. This rate of ovarian hyperstimulation syndrome at first seems particularly high. However, it should be recognized that all of the women had polycystic ovaries and were young, with an average age of 33.1 years. Both of these factors significantly increase the risk of developing ovarian hyperstimulation syndrome. Indeed, two previous studies examining the outcome of IVF in women with polycystic ovaries reported rates of moderate or severe ovarian hyperstimulation syndrome that were very similar to those in the present study: 10.5% and 10.3%. Although five women in the current study were classified as having severe ovarian hyperstimulation syndrome, only one required hospital admission, and another had drainage of her ascites as an outpatient procedure.

From 107 IVM cycles, 25 infants were born (seven multiple live births), and from an equal number of IVF cycles, 39 infants (ten multiple live births) were delivered. There was one set of triplets born in each treatment group. There were no reported cases of congenital abnormality in the infants. It is vital that data be collected on infants born after IVM treatment to allow full assessment of this new technology. We are currently engaged, in collaboration with pediatricians, in a prospective trial assessing the normality and neurodevelopmental development of IVM children conceived in our center. Results will be reported in due course, but at present IVM infants appear no different than those conceived through IVF.
REFERENCES


Address reprint requests to: Tim J. Child, MD, 25 Mill Lane, Oxford OX3 0QB, United Kingdom; E-mail: timothychild@yahoo.com.

Received February 15, 2002. Received in revised form April 24, 2002. Accepted May 2, 2002.
Appendix 12


Contribution by SJ Phillips

Manuscript writing and editing

Citation Metrics

Google Scholar: 0
Scopus: 0
Fertility Preservation in Premature Ovarian Insufficiency (POI) Secondary to FSH Receptor Gene (FSHR) Mutation: Is There a New Hope?

Pierre-Antoine Pradervand1,2, Roland Antaki1,2, Simon Phillips1,2, C. Sylvestre1,3, Zaki El Haffaf2, Ariane Godbout1,2, Isaac Jacques Kadoch1,2, Pierre Lehmann1,4

1OVO Fertility, OVO Clinic, Montreal, Canada
2Assisted Procreation Clinic, Centre Hospitalier de l’Université de Montréal (CHUM), Montreal, Canada
3Division of Reproductive Endocrinology and Infertility, Centre Hospitalier Universitaire (CHU) Ste-Justine, Montreal, Canada
4Department of Obstetrics and Gynecology, Hôpital Sacré Cœur de Montréal, Montreal, Canada
Email: papradervand@laфонdation.чh


Received: August 17, 2017
Accepted: October 20, 2017
Published: October 23, 2017

Copyright © 2017 by authors and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Abstract
A 19 years old patient with primary amenorrhea was referred to our center. Based on discrepancy between high follicle stimulating hormone (FSH) level and normal ovarian reserve parameters, follicle stimulating hormone receptor (FSHR) mutation was screened. The patient was homozygous in exon 6 of the FSHR gene for the new variant c.479 T > C and predicted to result in an aminoacid substitution p.Ile160Thr. One year later, her anti-müllerian hormone (AMH) level inexplicably decreased. Oocyte vitrification was thus offered for fertility preservation. After 17 days of recombinant follicle stimulating hormone (recFSH) (900 IU daily), no follicular growth was seen and estradiol levels remained low. In vitro maturation (IVM) was then suggested. Ten oocytes were successfully vitrified.

Keywords
FSH Receptor Mutation, Fertility Preservation, in Vitro Maturation, AMH, Primary Ovarian Insufficiency

1. Introduction
Primary ovarian insufficiency patients represent a major challenge for obstetricians and gynecologist in terms of fertility management. Even if it is rare, when POI is combined with a FSHR mutation, helping patient in her family planning looks almost impossible. If POI is most often of unknown etiology, the incidence
of family-related cases can achieve a percentage from 4% to 31%, suggesting a genetic etiology in a large proportion of patients [1] [2].

In 1995, Aittomäki et al. observed a particular Finnish population presenting with elevated gonadotropin levels and primary amenorrhea but a normal karyotype, ending in a POI [3]. Associated with streaks ovaries, this condition is called ovarian dysgenesis (ODG). Actually, Aittomäki et al. linked for the first time the ODG to a special FSH receptor (FSHR) mutation. They reported that a C566T transition in exon 7 of FSHR, predicting an Ala to Val substitution at residue 189 in the extracellular ligand-binding domain, was congruent with the ovarian dysgenesis observed in the Finnish population for homozygous women. More precisely, the locus segregating the disease can be mapped on chromosome number 2p, which corresponds to the locus for both FSHR and luteinizing hormone receptor (LHR) [4]. Other research in the western countries couldn’t find the same mutation in women with the same phenotype, suggesting that the finish case was very peculiar [5]. Beau et al. yet, identified another FSHR mutation in a woman with a secondary amenorrhea, elevated gonadotropins, a subnormal estrogenic activity and normal ovaries, a different phenotype than the ODG [6]. Since then, a few FSHR mutation studies were reported in the literature, and most of them highlight the link between FSHR mutation or polymorphism and an ovarian dysfunction that could present under different forms [4] [7].

To date, when such a mutation was suspected, ovarian biopsy was often suggested as the diagnostic tool and virtually no fertility treatment could be offered, but we now seem to have more to offer to these patients.

2. Case Presentation

A 19 years old patient with primary amenorrhea was referred to our center. She measured 166cm and weighed 57 kgs (body mass index 21.8). She had moderately developed secondary sex characteristics interesting particularly breasts, and diminished sized ovaries and uterus. She had normal axillary and pelvic hair, and had no morphotypic abnormalities. At this time, the antral follicular count (AFC) was 16 (8 + 8) and the anti-müllerian hormone (AMH) was 1.59 ng/ml.

Based on a discrepancy between high early follicular phase FSH level (72.5 IU/L) (ref. range 3.1 - 7.9 IU/L) and normal other ovarian reserve parameters, such as AMH levels and AFC, a FSHR mutation was suspected and searched for. A genetic screening was also performed for Fragile X Mental Retardation 1 gene (FMR1) premutation and for her karyotype. We furthermore completed an investigation of the classic auto-immune and endocrinologic causes of POF.

We found no FMR1 mutation or premutation and the karyotype was 46XX. The biological investigations were all normal. However, the patient was homozygous in exon 6 of the FSHR gene for a variant defined as c.479T > C and predicted to result in the amino acid substitution p.Ile160Thr. Her parents were non-consanguineous French Canadians and were carrying the same mutation.
They were healthy and the mother had no complications during her pregnancy or delivery.

One year later, her AMH level decreased from 1.59 to 0.62 ng/ml and her AFC from 16 to 9 for unexplained reason, so that a fertility preservation using oocyte vitrification was offered. Being aware of the FSHr mutation, we started with a stimulated cycle nonetheless. After 17 days of 900 IU daily doses of recombinant FSH (recFSH), no follicular growth was seen and estradiol levels remained low. Therefore an in vitro maturation (IVM) was done, with the use of subcutaneous hCG (10,000 IU) 34 hours before the ovarian function.

Three Further IVM cycles were carried out to maximize the number of vitrified mature oocytes. She firstly underwent five days of stimulation with daily doses of recFSH (300 IU) and recombinant luteinizing hormone (recLH) (300 IU) before the ovulation induction.

In vitro maturation was carried out using the following technique: follicular fluid was aspirated from small follicles (between 3 mm and 8 mm), the fluid was assessed by the embryologist using a 22 µm filter to maximise oocyte retention. Once the oocyte collection was completed, immature oocytes were washed using Global Total (IVFonline, Canada) and then transferred into the prepared IVM media. IVM media was made up on the morning of the oocyte collection, one 75 IU ampoule of Repronex (Ferring, Canada) was diluted and using to produce a 0.75 IU solution for the oocyte culture.

Immature oocytes were cultured for 25 hours in the maturation media and then assessed for cumulus expansion and extrusion of the first polar body; this was facilitated by sliding the oocyte. Sliding refers to visualizing the oocyte in a very thin layer of media created by tipping the dish gently to the side leaving the oocyte to flatten in the dish. Oocytes judged to be mature were denuded of their cumulus cells using recombinant hyaluronidase (Cumulase, Origio) and subjected to ICSI. Immature oocytes were further cultured for an additional 24 hours and assessed for maturation at which time they were denuded and ICSI was performed. Following ICSI, oocytes were transferred into our standard culture media system (Global, IVFonline, Canada).

In this 19 years old patient with a hypergonadotropic hypogonadism and a primary amenorrhea, we retained the diagnostic of FSHr-linked POI, taking in account the sudden diminution of her ovarian reserve.

With the fertility cryopreservation by oocyte vitrification, a total of 8 metaphase II oocytes (24 hours post IVM) and 2 metaphase I oocytes (post oocyte retrieval) were successfully vitrified and cryopreserved. A total of 3 IVM cycles were necessary for the obtention of a total of 10 oocytes.

3. Discussion

To our knowledge, this is the first reported successful fertility preservation by combining oocyte-IVM and vitrification for a POI patient. The main purpose of our case report is thus to suggest an opportunity for fertility preservation using
IVM in the management of patients with confirmed POI in the frame of a FSHR mutation. Gryenberg et al. already mentioned that there is a place for IVM in patients with ovarian resistance to FSH. They reported two cases of live birth after IVM in such a patient [8]. Even if their FSHR status was not specified, these successes are another argument in favor of IVM for POI patients with FSHR mutations. Our enthusiasm should probably be tempered by the fact that our patient is still far from delivery, but considering the number of cryopreserved eggs (a total of 10 eggs) and her young age, her predicted probability of having one child is over 60%, based on the work of Doyle et al. [9].

Being able to obtain mature oocytes in this clinical context is not a surprise. The pathway that prevents germinal vesicle (GV) and oocyte maturation is well characterized to comprise signaling from the granulosa cells (GC) that ultimately keeps cAMP high in the oocyte until communication with the GC is lost. In vivo this loss of communication normally happens because of the LH surge. But physical removal of GV oocytes from the follicle, as in our situation, has the same effect, lowering oocyte cAMP, and thus triggering oocyte maturation. This approach is used routinely experimentally to trigger oocyte maturation in many mammalian systems. Thus, it is no surprise that, even in the context of altered FSH signaling, any oocytes that managed to make it to a threshold size were capable of resuming meiosis [10] [11] [12].

Before aiming for fertility preservation, one should first obtain the correct diagnosis of POI and, possibly, more precisely the rare condition of FSHR mutation in order to anticipate an ulcerior ovarian failure in these unusual patients with amenorrhea. As for our patient, the diagnosis of POI was first suspected when a primary amenorrhea was associated with a hypergonadotropic hypogonadism, a normal AFC and a normal AMH. Such situations were formerly investigated with invasive techniques like an ovarian biopsy [13], in the certainty of a negative screening for FMR1 mutation or permutation [14] and a normal karyotype [15], trying to characterize a histological anomaly that could explain the clinical presentation. Of course, the possibility of genetic testing and the potential association of genetic mutation with POI open a new window of opportunity in the diagnosis and the support of these patients [16] [17]. In our case, no ovarian biopsies were available, thus no correlation was possible between the mutated receptor function and the ovarian histology.

Beau et al. have already and for the first time mentioned the reported missense mutation of our patient in 1998. It has to be clarified that their patient was found to carry a compound heterozygotic mutation affecting the FSHr gene and resulting in an Ile160Thr and an Arg573Cys substitutions. These substitutions were located, respectively, in the extracellular domain and in the third intracellular loop of the receptor. Their patient presented with another phenotype as she had secondary amenorrhea and normal sized ovaries [6] [18]. Also, Binder et al. [19] found that heterozygotic mutation Ile160Thr was carrying in 5.6% - 6.1% of infertile patients. But our patient is the first reported case of a homozygosity for the variant c.479T > C, suggesting that this condition could also lead to a POI.
Of course, amenorrhea or even POI are not obligatorily caused by a FSHR mutation. Achrekar et al. screened the entire FSHR gene in a population of Indian women with primary amenorrhea and found none of the known mutations. They identified a particular polymorphism that was more often present in women with elevated gonadotropins [20].

More recently, Desai et al. summarized the known FSHR mutation, their effect on the receptor function and the repercussion on the reproductive system for the affected woman [4]. This work suggests that the mutations are activating or inactivating. Inactivating mutations impair either the extracellular domain of the FSHR, resulting in the diminution of its expression at the ovarian cell surface, its ability to bind the ligand being not affected, or the affinity of the receptor to the ligand [4] [21]. In both situations, the formation of the receptor-ligand complex or the FSH signals transduction pathway would be altered ending to a diminution of the FSH induced cAMP production. But, in case of a diminished affinity, a higher gonadotropin concentration could partially solve the problem, which is not the case if the expression itself is impaired. If activating, the mutation was related to OHSS or even spontaneous OHSS.

Being convinced that IVM is worth considering as fertility preservation in young patients with POI, another interesting approach suggested by Suzuki et al. deserves attention. They report ovarian tissue cryopreservation by vitrification, followed by in vitro activation (IVA) of dormant follicles in POI patients with residual ovarian follicles. After activation, pieces of ovarian tissue are grafted back in the mesosalpinx and stimulated to obtain mature follicles. Out of their initial 37 patients, 20 had residual follicles. 9 of the 20 showed a follicular growth as a response to the post graft stimulation, and 24 oocytes were collected from 6 patients. Finally, 2 term pregnancies were obtained from these oocytes [22].

4. Conclusion

POI patients secondary to FSHR mutation are probably underdiagnosed due to the lack of readily available testing. FSHR mutation should be considered in patients with normal-high ovarian reserve (AMH and AFC) and discrepant FSH/LH results. We suggest that genetic analysis and further diagnosis precision should be more often investigated in these situations.

New hope is arising for patients carrying the FSHR gene mutation with POI, since we and others are showing encouraging results of fertility preservation for these patients. Being the first to report a case of successful oocyte vitrification following IVM in a patient with FSHr mutation, we call for further studies to assess this original approach and improve its success.

Acknowledgements

The authors gratefully thank the OVO R & D unit for their technical help.

P.-A. Pradervand thanks the SICPA foundation and the Société Académique Vaudoise for their support.
References


Appendix 13


Contribution by SJ Phillips

  Concept
  Data collection
  Data analysis
  Manuscript writing and editing

Citation Metrics

  Google Scholar:  67
  Scopus:  49
  Web of Science:  43
Working to eliminate multiple pregnancies: a success story in Québec

F Bissonnette a,*, SJ Phillips a, J Gunby b, H Holzer c, N Mahutte d, P St-Michel e, IJ Kadoch a

a OVO Fertility, 8000 Boulevard Decarie #100, Montréal, Québec, Canada H4P 2S4; b Canadian ART Register, 2534 Cavendish Drive, Burlington, Ontario, Canada L7P 4E4; c McGill Reproductive Centre, 687 Pine Avenue West, Montréal, Québec, Canada H3A 1A1; d The Montreal Fertility Centre, 5252 de Maisonneuve Blvd West, Suite 220, Montréal, Québec, Canada H4A 3S5; e Procrea Cliniques, 1361, avenue Beaumont, #301, Mont-Royal, Québec, Canada H3P 2W3

* Corresponding author. E-mail addresses: f.bissonnette@cliniqueovo.com, fbis@videotron.ca (F Bissonnette).

Dr. François Bissonnette is the medical director of OVO, a fertility clinic in Montreal, and a professor in the department of Obstetrics-Gynecology at the University of Montreal. He completed his speciality training in obstetrics and gynecology in 1980 and was certified by the Royal College of Physicians and Surgeons of Canada in 1985. He was subsequently trained in reproductive endocrinology and infertility at the University of Louisville, Kentucky. He is currently the director of the Canadian ART Registry and has been President of the Canadian Fertility and Andrology Society in 2009.

Abstract  In August 2010, the provincial government of Québec, Canada introduced funding of assisted reproduction treatment through the provincial health programme. Alongside this benefit, legislation was introduced to control assisted reproduction treatment activities in the province, including restrictions on the number of embryos that could be transferred in any one cycle. The aim of the programme was to transfer a single embryo in every cycle; multiple embryos could be transferred under suboptimal conditions but required physician justification. In the first 3 months of this programme, 1353 cycles of IVF were performed in five Québec assisted reproduction centres, with an overall clinical pregnancy rate of 32% per embryo transfer and 50% of transfers used elective single-embryo transfer (eSET). The multiple-pregnancy rate was only 3.7% per clinical pregnancy. In 2009, prior to the introduction of the programme, eSET was used in only 1.6% of embryo transfers, resulting in a multiple-pregnancy rate of 25.6%. These data demonstrate that providing provincially funded assisted reproduction treatment created an environment in which the aggressive use of eSET was not only possible, but also rapidly implemented. The result was a dramatic drop in multiple-pregnancy rates, approaching those for natural pregnancies.

Introduction  Huge advances have been seen in IVF success rates over the more-than 30 years that this treatment has been applied clinically. Improvements in laboratory practices, ovarian stimulation protocols, cryopreservation techniques and genetic aspects of treatment have all served to increase implantation and live-birth rates. However, despite these
advances, one major drawback of IVF has remained constant: the disproportionately high rates of multiple pregnancies, including high-order multiple pregnancies, and the associated risks seen with such outcomes.

In Canada, professionals working in the field of assisted human reproduction meet under the auspices of the Canadian Fertility and Andrology Society (CFAS). For the last 11 years, the CFAS has managed a registry of assisted reproduction cycles performed across the country. Participation in the Canadian ART Register (CARTR) is not mandatory but all Canadian assisted reproduction clinics participate and provide funding for this initiative. The registry allows Canadian clinics to monitor the outcome measures associated with treatment in the country. Although only aggregate data are released to participants and the general public, the CARTR outcome-improvement committee has confidential access to clinic-specific data, permitting them to identify and offer help to clinics whose results fall below the national standard.

Looking at published data from 2005, it is clear that North America has the greatest problem with multiple births from assisted reproduction treatment; Canada and the USA topped the statistics with >30% multiple-birth rates, whereas the lowest rates were seen in countries with state coverage for assisted reproduction treatment, such as Sweden (6%), Australia/New Zealand (14%) and Belgium (13%) (Assisted Human Reproduction Canada, 2011).

Data from CARTR shows the progression of the multiple-birth rate compared with the live-birth rate in Canada from 2001 to 2008 (Figure 1) (Gunby et al., 2010). The live-birth rate has improved gradually over the years, but the multiple-pregnancy rate fluctuates consistently around the 30% rate. Although the use of elective single-embryo transfer (eSET) for assisted reproduction in Canada has been slowly increasing, by 2009 it remained at a very low rate (Figure 2). The low use of eSET can be attributed to the fact that, when patients are paying for their treatment, the pressure to achieve a pregnancy with as few attempts as possible becomes a driving force for both the patient and treating physician. In the absence of strict guidelines, a clinic may be tempted to transfer more embryos to improve its pregnancy rate.

Table 1 shows comparative data for 2009 between Québec and the rest of Canada, according to CARTR. It is clear that, although the multiple-pregnancy rate in Québec was slightly lower than the rest of Canada (25.6% versus 29.3%), the use of eSET was more popular in the rest of Canada (6.5% of embryo transfers) than in Québec (1.6%).

A large number of studies have discussed the potential benefits of eSET and the financial issues, comparing ongoing costs associated with multiple pregnancies and repeated IVF cycles due to reduced pregnancy rate when using eSET (Bromer et al., 2011; Fauque et al., 2010; Gerris, 2009; McLer- non et al., 2010; Moustafa et al., 2008; Van Peperstraten et al., 2008).

In Canada, health care is the responsibility of the individual provinces; thus, each province determines healthcare coverage. In Québec, the provincial government decided to include assisted reproduction treatment for infertility under the provincial health plan starting on 5 August 2010. The policy provides for any woman of reproductive age to have up to three cycles of IVF with ovarian stimulation or up to six cycles of natural or modified natural cycle IVF at no charge; this applies whether the cycles are performed within a hospital unit or a privately owned facility. A cycle counts once an embryo transfer has been performed. Patients who have excess embryos cryopreserved following an IVF cycle are obliged to have those embryos thawed for transfer before embarking on another ovarian stimulation cycle, but frozen—thawed embryo transfers do not count against their three attempts.

In exchange for this coverage, the government imposed a limit on the number of embryos that could be transferred in any one cycle. In effect, the law states that only one embryo should be transferred in either a fresh or frozen IVF cycle. However, an option to transfer up to two embryos in a woman aged 36 years or younger and up to three embryos (including no more than two blastocysts) in a woman aged 37 years or older is available, but the physician must justify his decision (An Act Respecting Clinical and Research Activities related to Assisted Procreation, 2010).

The purpose of this study is to report on the outcomes (pregnancy rates and multiple-pregnancy rates) of IVF

---

**Figure 1** Change in multiple-birth rate compared with live-birth rate in Canada between 2001 and 2008 (data from the Canadian ART Register).

**Figure 2** Change in percentage of embryo transfers using elective single-embryo transfer in Canada between 2001 and 2009 (data from the Canadian ART Register).
cycles in Québec from the first 3 months of provincially funded assisted reproduction treatment.

Materials and methods

All IVF cycles started in Québec assisted reproduction centres from 5 August to 5 November 2010 were recorded. The average patient age was 37 years, with patients ranging from 22 to 46 years old. Each of the five centres applied its own standard protocols for ovarian stimulation and laboratory procedures. Ovarian-stimulation protocols, including long gonadotrophin-releasing hormone (GnRH) agonist, short GnRH agonist and GnRH antagonist, were selected based on physician preference and patient characteristics. Egg retrieval was performed 34.5–36 h after the administration of human chorionic gonadotrophin, based on individual clinic policy. Insemination was performed using standard IVF or intracytoplasmic sperm injection. Embryo culture was performed using Cook Sydney IVF media (Cook, Canada) in all centres.

Embryo transfer was performed in all centres under ultrasound guidance on either day 2, day 3 or at the blastocyst stage depending on cycle-specific characteristics. Two clinics performed the majority of their embryo transfers at the blastocyst stage, one clinic transferred equally on day 2, day 3 and at the blastocyst stage, one clinic performed the majority of their transfers on day 3 and one clinic performed transfers on both day 3 and at the blastocyst stage. Each clinic applied their own internal policies for embryo quality and selection as well as when it was judged pertinent to transfer more than one embryo. In general, the patient’s age and quality of the embryos were primary factors in any decision to transfer multiple embryos as well as previous IVF history. Embryo-quality selection was applied when selecting suitable embryos for cryopreservation based on individual clinic protocols. Oocyte and embryo development parameters were strictly applied in order to eliminate embryos with very low potential.

Pregnancy was assessed by serum human chorionic gonadotrophin concentration 15 days after egg retrieval and clinical pregnancy was determined by ultrasonographic evidence of intrauterine fetal heartbeat at 8 weeks’ gestation.

Results

From 5 August to 5 November 2010, a total of 1353 IVF cycles were started in Québec centres, 1276 cycles had egg retrieval and 1103 cycles resulted in embryo transfer. For comparison, only 517 IVF cycles were started in the same time period in 2009.

Overall, the clinical pregnancy rate was 32% and eSET was used in 50% of embryo transfers (Table 2). In the younger patient group (<35 years old), eSET was used in 79% of embryo transfers and resulted in a clinical pregnancy rate of 40%. There were a total of only 13 twin pregnancies and no triplet pregnancies. All twin pregnancies resulted from the transfer of two embryos, either elective double-embryo transfer (eDET) where two embryos were selected from more than two available embryos or non-elective DET where only two embryos were available for transfer. This equates to a multiple-pregnancy rate of 3.7%.

Table 3 describes the outcomes based on the number of embryos transferred, grouped by female age. For all age groups combined, pregnancy rates per embryo transfer were highest with eSET (38%), compared with non-elective SET (21%), eDET (32%), non-elective DET (21%) and transfer of more than two embryos (25%). Twin pregnancy rates were 0% for eSET and non-elective SET, 13% for eDET, 17% for non-elective DET and 0% for transfer of more than two embryos.

Overall, 42% of patients had supplementary embryos cryopreserved following their IVF cycle, with an average of 3.4 embryos per patient. For patients <35 years of age, 60% of patients had cryopreservation, with an average of 3.7 embryos per patient (Table 4).

A summary of the results seen in Québec prior to and after the implementation of the programme can be seen in Table 5.

Discussion

Multiple pregnancies are, undoubtedly, the major negative side effect of assisted reproductive therapies and, in countries where patients are responsible for the total cost of treatment, the multiple-pregnancy rate is often higher. An assumption that the transfer of multiple embryos will substantially increase the chance of pregnancy and, therefore, reduce the number of expensive attempts to achieve the desired live birth, places pressure on patients who, in turn, transfer this pressure to healthcare professionals charged with their reproductive care.

In Québec, the government decided that provincially funded assisted reproduction was the correct direction to take. This policy provides access to treatment for all members of society, regardless of income and resources. In addition, by imposing limitations on the number of embryos that

<table>
<thead>
<tr>
<th>Variable</th>
<th>Québec</th>
<th>Canada except Québec</th>
<th>Canada</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF cycles</td>
<td>1831</td>
<td>8524</td>
<td>10,355</td>
</tr>
<tr>
<td>Embryo transfers</td>
<td>1618</td>
<td>7417</td>
<td>9035</td>
</tr>
<tr>
<td>Clinical pregnancies (rate per ET)</td>
<td>692 (42.8)</td>
<td>3164 (42.7)</td>
<td>3856 (42.7)</td>
</tr>
<tr>
<td>eSET (rate per ET)</td>
<td>26 (1.6)</td>
<td>484 (6.5)</td>
<td>510 (5.6)</td>
</tr>
<tr>
<td>Multiple pregnancies (rate per pregnancy)</td>
<td>177 (25.6)</td>
<td>927 (29.3)</td>
<td>1104 (28.6)</td>
</tr>
</tbody>
</table>

Values are n or n (%). eSET = elective single-embryo transfer; ET = embryo transfer.
can be transferred in any one cycle, a reduction in multiple-pregnancy rates should occur. Although the policy has the implicit aim of single-embryo transfer in as many cases as possible, the Québec government had been listening to clinicians who stated that there are situations that justify the transfer of more than one embryo. The costs associated with the ongoing care of multiple pregnancies, both during the pregnancy itself and for the care of premature infants, possibly with life-time increased health costs, can be exorbitant. By reducing the number of multiple pregnancies and multiple births, the subsequent reduction in associated health costs can be used to pay for the assisted reproduction treatment. This appears to be a more positive use of provincial health funds since it is aimed at producing healthy babies rather than needing to support babies with health issues.

Although other countries have introduced similar policies, the authors believe that this case demonstrates the quickest realization of the aim of almost eliminating multiple pregnancies in response to state-sponsored IVF. Québec fertility specialists are very motivated to make this programme work and have adopted stricter criteria than those imposed by government. In Québec, the multiple-pregnancy rate was reduced from 25.6% to only 3.7% by using 50% eSET, compared with only 1.6% eSET previously, in only 3 months. The correct application of the law can be demonstrated by the fact that in those cycles where two embryos were transferred the twin rate is very low and that there are no triplets from cycles where three embryos were transferred. This indicates that the appropriate selection of patients needing more than eSET can be achieved if clinicians apply careful criteria in patient selection. If this is

### Table 2 Results from IVF cycles started in the first 3 months of assisted reproduction treatment funding in Québec.

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;35 Years</th>
<th>35–39 Years</th>
<th>≥40 Years</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycles started</td>
<td>522</td>
<td>491</td>
<td>340</td>
<td>1353</td>
</tr>
<tr>
<td>Egg retrievals</td>
<td>500</td>
<td>458</td>
<td>318</td>
<td>1276</td>
</tr>
<tr>
<td>Embryo transfers</td>
<td>443</td>
<td>388</td>
<td>272</td>
<td>1103</td>
</tr>
<tr>
<td>Clinical pregnancies (rate per ET)</td>
<td>177 (40)</td>
<td>124 (32)</td>
<td>47 (17)</td>
<td>348 (32)</td>
</tr>
<tr>
<td>eSET (rate per ET)</td>
<td>348 (79)</td>
<td>175 (45)</td>
<td>33 (12)</td>
<td>556 (50)</td>
</tr>
<tr>
<td>Multiple pregnancies (rate per pregnancy)</td>
<td>2 (1.1)</td>
<td>7 (5.6)</td>
<td>4 (8.5)</td>
<td>13 (3.7)</td>
</tr>
</tbody>
</table>

Values are n or n (%). eSET = elective single-embryo transfer; ET = embryo transfer.

### Table 3 Results from elective and non-elective single- and double-embryo transfers in IVF cycles started in the first 3 months of assisted reproduction treatment funding in Québec.

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;35 Years</th>
<th>35–39 Years</th>
<th>≥40 Years</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>eSET</td>
<td>348</td>
<td>175</td>
<td>33</td>
<td>556</td>
</tr>
<tr>
<td>eSET clinical pregnancies (rate per ET)</td>
<td>144 (41)</td>
<td>61 (35)</td>
<td>8 (24)</td>
<td>213 (38)</td>
</tr>
<tr>
<td>Non-elective SET</td>
<td>48</td>
<td>54</td>
<td>49</td>
<td>151</td>
</tr>
<tr>
<td>Non-elective SET clinical pregnancies (rate per ET)</td>
<td>15 (31)</td>
<td>13 (24)</td>
<td>3 (6)</td>
<td>31 (21)</td>
</tr>
<tr>
<td>eDET</td>
<td>17</td>
<td>74</td>
<td>81</td>
<td>172</td>
</tr>
<tr>
<td>eDET clinical pregnancies (rate per ET)</td>
<td>9 (53)</td>
<td>27 (36)</td>
<td>19 (23)</td>
<td>55 (32)</td>
</tr>
<tr>
<td>eDET twin pregnancies (rate per ET)</td>
<td>1 (11)</td>
<td>4 (15)</td>
<td>2 (11)</td>
<td>7 (13)</td>
</tr>
<tr>
<td>Non-elective DET</td>
<td>30</td>
<td>72</td>
<td>70</td>
<td>172</td>
</tr>
<tr>
<td>Non-elective DET clinical pregnancies (rate per ET)</td>
<td>9 (30)</td>
<td>18 (25)</td>
<td>9 (13)</td>
<td>36 (21)</td>
</tr>
<tr>
<td>Non-elective DET twin pregnancies (rate per ET)</td>
<td>1 (11)</td>
<td>3 (17)</td>
<td>2 (22)</td>
<td>6 (17)</td>
</tr>
<tr>
<td>&gt;Two embryos transferred</td>
<td>0</td>
<td>13</td>
<td>39</td>
<td>52</td>
</tr>
<tr>
<td>Clinical pregnancies &gt; Two embryos</td>
<td>0</td>
<td>5 (38)</td>
<td>8 (21)</td>
<td>13 (25)</td>
</tr>
</tbody>
</table>

Values are n or n (%). DET = double-embryo transfer; cDET = elective double-embryo transfer; eSET = elective single-embryo transfer; ET = embryo transfer; SET = single-embryo transfer.

*All singleton pregnancies.

### Table 4 Proportion of patients having embryo cryopreservation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;35 Years</th>
<th>35–39 Years</th>
<th>≥40 Years</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with embryo cryopreservation (n(%))</td>
<td>286 (60)</td>
<td>144 (37)</td>
<td>57 (21)</td>
<td>487 (43)</td>
</tr>
<tr>
<td>Embryos cryopreserved per patient (mean)</td>
<td>3.7</td>
<td>3.2</td>
<td>2.7</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Table 5 Summary of impact of the state coverage of IVF cycles in Québec.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo transfers performed using eSET</td>
<td>1.6</td>
<td>50.4</td>
</tr>
<tr>
<td>Clinical pregnancy rate</td>
<td>42.8</td>
<td>31.6</td>
</tr>
<tr>
<td>Multiple-pregnancy rate</td>
<td>25.6</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Values are %. eSET = elective single-embryo transfer.

done it means that legislative terms such as those created in Québec, whereby the clinician has some flexibility to transfer multiple embryos if deemed truly necessary, can be successfully applied. It is clear that buy-in by the clinics is necessary with a programme such as this where the option to transfer more than one embryo is permitted. Whilst this option is important to allow for those cases where multiple embryo transfer is required to maintain pregnancy potential for the patient, it opens the door to potential abuse; if only one clinic in the state continues to unnecessarily transfer multiple embryos, the aim of the programme to reduce multiple pregnancies will not be achieved.

Furthermore it is noted that there is a higher-than-usual rate of cycles with no embryo transfer. This may be explained by the fact that all the centres applied their own internal policies on embryo selection criteria but that the criteria were strictly applied, so that the elimination of suboptimal embryos led to a higher cancellation rate. This was carried out in an attempt to maintain clinical pregnancy rates with eSET and ensure that only suitable embryos were considered for cryopreservation.

The major concern when using eSET liberally is that the pregnancy rate will be negatively affected. The implementation of this programme saw the overall clinical pregnancy rate fall from 42.8% to 31.6%; however, it is important to remember that nearly 50% (48.7%) of patients under 40 years of age had at least one embryo cryopreserved following their fresh IVF cycle, with an average of 3.5 embryos each. Although this may seem a high proportion of patients having a high number of embryos cryopreserved, it is believed that this relates to the fact that when transferring only one embryo the chance that at least one other embryo will be available for cryopreservation increases.

With the improvement of outcomes in cryopreserved embryos, especially with the introduction of vitrification, it is logical to use the cumulative pregnancy rate or cumulative live-birth rate per initiated cycle, combining results from transfer of fresh and frozen embryos, as the standard measure of a patient’s chances for a baby. Certainly, in this programme, where the use of cryopreserved embryos is included and does not reduce the patient’s number of funded cycles, it is clear that the cumulative pregnancy rate and cumulative live-birth rate must be considered.

Further analysis will need to be performed as more data becomes available to ensure that the patterns demonstrated by the initial analysis remain constant and also to assess the cumulative pregnancy rate once sufficient—thawed embryo transfers have been performed. It is believed that once this data is included the cumulative pregnancy rate for patients under 40 years of age will demonstrate that the policy of ‘one embryo at a time’ can be successfully implemented in the right environment.

Acknowledgements

The authors would like to thank the clinical teams from all five Québec assisted reproduction treatment centres for their hard work in achieving these results in such a short period of time.

References


Declaration: The author reports no financial or commercial conflicts of interest.

Received 21 January 2011; refereed 29 April 2011; accepted 10 May 2011.
Appendix 14

Velez MP, Kadoch IJ, Phillips SJ, Bissonnette F. Rapid policy change to single-embryo transfer while maintaining pregnancy rates per initiated cycle. RBMOnline. 2013. 26:506-511

Contribution by SJ Phillips

Concept
Data collection
Manuscript writing and editing

Citation Metrics

Google Scholar: 18
Scopus: 10
Web of Science: 6
Rapid policy change to single-embryo transfer while maintaining pregnancy rates per initiated cycle

MP Vélez a,b,*, I-J Kadocha,b, SJ Phillips a, F Bissonnette a,b

a OVO Clinic, 8000 boul Decarie, Suite 100, Montréal, Québec, Canada H4P 2S4; b Département d’obstétrique-gynécologie, Université de Montréal, Centre Hospitalière de l’Université de Montréal. Hôpital Saint-Luc, Clinique de Procréation Assistée, 888 boulevard de Maisonneuve Est, Suite 200, Montréal, Québec, Canada H2L 4S8
* Corresponding author  E-mail address: mdp.velez.gomez@umontreal.ca (MP Vélez).

Dr Maria P Vélez is an obstetrician and gynaecologist with a Masters in epidemiology. She is completing a fellowship in reproductive endocrinology and infertility at the University of Montreal, and a PhD in public health/epidemiology at the same institution. Her research interests include the clinical impact of publicly funded IVF programmes and elective single-embryo transfer, as well as the study of different indicators and determinants of reproductive health such as anti-Müllerian hormone and several environmental contaminants.

Abstract  Public financing of IVF aims at increasing access to treatment while decreasing the expenses associated with multiple pregnancies. Critics argue that it is associated with lower pregnancy rates. This study compared cycles performed during 2009 (before implementation of Quebec’s public IVF programme; period I) to those performed in the year following implementation (period II) in a single IVF centre. First fresh cycles in period I (499 women) and first fresh cycles (815 women) along with their corresponding first vitrified—warmed transfer (271 women) in period II were evaluated. From period I to period II, single-embryo transfer increased from 17.3% to 85.0% (P < 0.001), multiple ongoing pregnancy rate decreased from 25.8% to 1.6% (P < 0.001) and ongoing pregnancy rate decreased from 31.9% to 23.3% (P = 0.001). During period II, the ongoing pregnancy rate per vitrified—warmed embryo transfer was 19.2%, leading to a cumulative ongoing pregnancy rate per initiated cycle of 29.7%, which was not different to the pregnancy rate per fresh cycle during period I (31.9%). To conclude, Quebec’s public IVF programme decreased multiple pregnancy rates while maintaining an acceptable cumulative ongoing pregnancy rate, a more precise outcome to evaluate the impact of public IVF programmes.

© 2013, Reproductive Healthcare Ltd. Published by Elsevier Ltd. All rights reserved.

KEYWORDS: assisted reproductive treatment, IVF, multiple pregnancy, public policy, single-embryo transfer

Introduction

Public financing of assisted reproductive technology is intended to increase access to fertility treatments by reducing the financial burden to patients. In counterpart, governments aim to reduce the health expenses associated with multiple pregnancies attributable to the use of assisted reproductive technology. Although comprehensive government initiatives are associated with greater utilization of fertility treatments and lower rates of multiple pregnancies (Bissonnette et al., 2011), critics of public financing argue that public programmes are also associated with lower
pregnancy rates (Gleicher, 2011). Many countries have implemented legislation to regulate assisted reproductive technology, encouraging the practice of single-embryo transfer (SET) through public funding (Maheshwari et al., 2011). In Canada, the only province that totally covers the costs of treatment is Quebec (Bissonnette et al., 2011). The subject of how many embryos is safe and proper to transfer and the best way to regulate this decision continues to generate debate and no unanimity has been reached to date (Gleicher, 2011; Johnson et al., 2011; Khalaf et al., 2011).

SET is the most effective approach to reduce the incidence of multiple pregnancies after assisted reproductive treatment (ESHRE, 2001). However, most of the studies evaluating the outcomes of SET have shown lower pregnancy rates when comparing with double-embryo transfer (DET; Pandian et al., 2009). Nonetheless, the principal weakness of these studies is not taking into account that most SET are not elective. Elective SET requires the selection of good prognosis patients and the transfer of the best high quality embryo (De Sutter et al., 2003). Studies that have compared elective SET with DET have not found differences in terms of pregnancy rates among both groups (De Sutter et al., 2003; Dhont, 2001; Gerris et al., 2004).

Moreover, in regards to health expenses, a prospective economic study demonstrated that the transfer of a single top-quality embryo is substantially cheaper than DET in women <38 years of age in their first IVF cycle (Gerris et al., 2004).

Public financing of assisted reproductive technology usually covers three or more treatment cycles. Therefore, to evaluate the effectiveness of these programmes, cumulative pregnancy rates per initiated cycle are more relevant than the pregnancy rates per fresh cycle (Thurin-Kjellberg et al., 2009). As part of this strategy, DET should be compared with SET including the resulting first cryopreserved embryo transfer.

Starting on 5 August 2010, all costs related to IVF have been covered by the Quebec Public Health Insurance Plan. The programme covers three stimulated cycles of IVF or up to six cycles in the case of modified natural cycles. The law states that only one embryo may be transferred at a time. Under very specific conditions (embryo quality and age) the law does allow for clinicians to transfer a maximum of two embryos if the woman is aged 36 and a maximum of three embryos or two blastocysts if the woman is aged 37 or over (Gouvernement de Québec, 2010). The objective of the programme was to reduce the number of multiple pregnancies resulting from IVF from 30% to 5% (Gouvernement de Québec, 2012).

OVO clinic is a private centre affiliated with the University of Montreal. The Quebec funding programme leaves the patient with the choice of going to a private or hospital-based centre with no difference in the cost to the couple.

The aim of the present study is to evaluate the impact of the Quebec public IVF programme with emphasis on a strict SET policy, in terms of utilization, pregnancy rates and multiple pregnancies comparing those IVF cycles performed in the OVO clinic during 2009 to those cycles performed during the first year following implementation of the new Quebec public IVF programme.

Materials and methods

Study population

Two time periods were compared. Period I includes information from IVF cycles performed at the OVO clinic during 2009, before implementation of the new public IVF programme. Period II comprises IVF cycles performed at OVO clinic during the first year following implementation of the new Public IVF programme (from 5 August 2010 to 4 August 2011). Excluded from the analysis are the 6 months between the two periods because the treated population changed considerably as the programme was announced and many couples decided to wait for gratuity.

OVO clinic is a leading centre in North America performing modified natural IVF (Kadoch et al., 2011) and this technique represents more than 25% of the IVF activity in this centre. Nonetheless, for the purpose of calculating time-limited cumulative pregnancy rates including the resulting first cryopreserved embryo transfer cycle (Daya, 2005), only the first cycles of stimulated IVF cycle during both periods, additional to the first cryopreserved cycle in period II, were analysed. Around 30% of the couples had previous undergone IVF cycles before the studied periods; nonetheless, the number of previous cycles did not differ between both periods. In addition, cycles resulting in the cryopreservation of all embryos were excluded from the analysis since their inclusion would underestimate the per cycle pregnancy rates (i.e. 13/512 (2.5%) during period I and 62/877 (7.1%) during period II; Yovich and Junk, 1999).

Stimulation protocol, oocyte retrieval and embryology procedures

Ovarian stimulation protocols, including long gonadotrophin-releasing hormone (GnRH) agonist, short GnRH agonist and GnRH antagonist, were selected based on physician preference and patient characteristics. The long GnRH agonist was mainly used during the first period (53.4%), while the GnRH antagonist was the main protocol prescribed during period II (52.2%). Egg retrieval was performed 36 h after the administration of human chorionic gonadotrophin. Insemination was performed using standard IVF or intracytoplasmic sperm injection when indicated. Embryo culture was performed using standardized procedures. Embryo transfer was performed under ultrasound guidance on day 3 or at the blastocyst stage depending on cycle-specific characteristics.

Cryopreservation

Embryo quality characteristics were applied when selecting suitable embryos for cryopreservation based on individual clinic protocols. Embryo development parameters were strictly applied in order to eliminate embryos with very low potential ( ALPHA-ESHRE, 2011; Steer et al., 1992). Vitrification was used for cryopreservation. Cleavage-stage vitrified–warmed embryos were transferred in 98.2% of the cases.
Outcomes

Pregnancy was assessed by serum human chorionic gonadotrophin (HCG) concentration 15 days after egg retrieval. Biochemical pregnancy was defined by HCG ≥25 IU/l; and clinical pregnancy was determined by ultrasonographic evidence of intrauterine fetal heart beat at 7 weeks of gestation (Zegers-Hochschild et al., 2009). Pregnancy rates were calculated on a per woman basis and defined as the proportion of women achieving pregnancy out of the total number who started treatment. Pregnancy rates after the first fresh IVF cycle performed during period I, a period where DET was prevalent, were compared with cumulative pregnancy rates of period II, a period where SET was prevailing. The time-limited cumulative pregnancy rate for period II was defined as the proportion of clinical pregnancies after the first fresh IVF cycle, including the resulting first vitrified–warmed embryo transfer cycle (Daya, 2005). Multiple pregnancies were defined according to the number of embryos with a positive heart beat. Cryopreserved embryos were defined as the number of surplus embryos available for cryopreservation.

Statistical analysis

Proportion comparisons were performed by chi-squared test or Fisher’s Exact test when indicated. Student’s t-test and ANOVA were used to compare means. A P-value <0.05 was considered to be statistically significant. Statistical analysis was performed using STATA version 10.0 (Stata Corporation, College Station, TX, USA).

This retrospective study was approved by OVO research and development scientific board on 4 January 2012.

Results

A total of 499 women started a cycle of stimulated IVF at OVO clinic during 2009 (period I), while 815 women started a cycle of stimulated IVF during the first year following implementation of the new Quebec government legislation (period II), representing an increase of 64% on utilization/access rates.

The age of the women was similar in both periods: mean ± SD, 35.2 ± 4.4 years for period I and 35.4 ± 4.4 years for period II. In addition, no differences were seen in the distribution of women per categories of age (Table 1). The proportion of oocyte retrievals was higher in period II, while no differences were seen on the number of mature eggs retrieved. Although the proportion of women having embryo transfer was similar between both periods, the mean number of embryos transferred during period I was higher than the cumulative number of embryos transferred during period II. The overall ongoing clinical pregnancy rate after the first fresh cycle of stimulated IVF was higher during period I than in period II (P = 0.001). Nonetheless, no difference was seen when period I was compared with the cumulative ongoing pregnancy rate of period II. After stratifying by categories of age, the difference between the fresh cycles was present only in women younger than 37 years, while no difference was seen in women aged 37 and older. In both categories of age, the CPR during period II was similar to the ongoing pregnancy rate per fresh cycle during period I.

Figure 1 shows the modalities of embryo transfer performed during both periods. SET increased during the second period (P < 0.001), while DET and the transfer of more than two embryos decreased (P < 0.001).

Table 2 presents clinical pregnancy rates according to the number of embryos transferred during both periods. After SET, the ongoing clinical pregnancy rate per embryo transfer was higher in period II compared with period I. In contrast, DET resulted in a lower ongoing pregnancy rate during period II compared with period I. No differences in clinical pregnancy rates per embryo transfer were found between both periods when more than two embryos were transferred.

Multiple pregnancies following the first fresh IVF cycle

The multiple pregnancy rates decreased considerably during period II. According to the modalities of embryo transfer, no multiple pregnancies were reported after SET in period I, while one monozygotic twin pregnancy was observed during period II (Table 3). In contrast, the prevalence of multiple pregnancies after DET decreased from period I to period II. Of note, during the first period very few pregnancies were the result of SET, with the majority issued from DET; in contrast, during the second period the vast majority of pregnancies resulted from SET (Table 3).

Cryopreserved embryos

Overall, the proportion of cryopreserved embryos per cycle started significantly increased from period I to period II (P < 0.001). Table 4 shows the proportion of women having cryopreserved embryos according to age. Younger women had a higher proportion of cryopreserved embryos than older women in both periods. Additionally, the number of cryopreserved embryos per patient was higher in period II compared with period I (Table 4).

Discussion

This study supports that the access to IVF, as measured by the number of cycles performed at OVO clinic after implementation of the public IVF programme, increased. It also finds that the proposed objective of the public IVF programme of reducing multiple pregnancies was met. Additionally, although the per cycle pregnancy rate after the first stimulated IVF cycle was lower in period II compared with period I, the cumulative pregnancy rate in period II (i.e. up to one IVF cycle, including the first resulting cryopreserved embryo transfer) was similar to the pregnancy rate per cycle during period I. Furthermore, the programme resulted in a higher proportion of women having cryopreserved embryos than in period I, a difference that can further impact on cumulative pregnancy rates.

Lack of public funding has a major role in the unequal access of women to clinically appropriate infertility treatment in Canada (Nisker, 2008). The proportion of infertile couples who had access to IVF treatment in the province
of Quebec before the public IVF programme has not been assessed in a formal manner. Data from this study clinic indicates that before the public funding only one-quarter of the infertile population had access to IVF and that this was mainly because of the cost of treatment. Utilization/access to fertility treatment is determined by factors such as the number of centres available, the cost to the individual, and distance and opening hours (Collins, 2002). In the year following implementation of the programme, the number of IVF clinics in Quebec, as well as their distance and opening hours was the same as before the public funding. Thus, the increase in access to IVF after the public programme was mainly through the impact on the cost of treatment for all women, with certainly greatest benefit to the least advantaged infertility patients.

Figure 1 Modalities of embryo transfer. SET = single-embryo transfer; DET = double-embryo transfer; >DET = more than two embryos transferred.

Table 1 Outcomes of stimulated IVF cycles performed at OVO clinic preceding and following Quebec’s public IVF legislation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Period I (499 women)</th>
<th>Period II (815 women)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First fresh cycle</td>
<td>First fresh cycle</td>
<td>First cryopreserved cycle</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37</td>
<td>333 (66.7)</td>
<td>530 (65.0)</td>
<td>NS a</td>
</tr>
<tr>
<td>≥37</td>
<td>166 (33.3)</td>
<td>285 (35.0)</td>
<td></td>
</tr>
<tr>
<td>Retrievals</td>
<td>446 (89.4)</td>
<td>764 (93.7)</td>
<td>0.004 b</td>
</tr>
<tr>
<td>Mature eggs</td>
<td>10.9 ± 6.2</td>
<td>11.0 ± 6.5</td>
<td>NS c</td>
</tr>
<tr>
<td>Transfers</td>
<td>423 (84.8)</td>
<td>666 (81.7)</td>
<td>0.001 d</td>
</tr>
<tr>
<td>Embryos transferred</td>
<td>2.11 ± 0.81</td>
<td>1.18 ± 0.44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ongoing pregnancies</td>
<td>159</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancy rate per cycle</td>
<td>31.9</td>
<td>23.3</td>
<td></td>
</tr>
<tr>
<td>Age &lt;37 years</td>
<td>136 (40.8)</td>
<td>151 (28.5)</td>
<td>0.002 e</td>
</tr>
<tr>
<td>Age ≥37 years</td>
<td>23 (13.9)</td>
<td>39 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>118 (74.2)</td>
<td>187 (98.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiple</td>
<td>41 (25.8)</td>
<td>3 (1.6)</td>
<td></td>
</tr>
</tbody>
</table>
| Values are n (%), mean ± SD or n. Period I = from 1 January to 31 December 2009; period II = from 5 August 2010 to 4 August 2011. NS = not statistically significant. 

* Chi-squared test.

* Period I compared with first fresh cycle in period II.

* Student’s t-test.

* Period I compared with cumulative data in period II.

Table 2 Clinical pregnancy rates according to the number of embryos transferred in first fresh cycles.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Period I</th>
<th>Period II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfers</td>
<td>423</td>
<td>666</td>
<td></td>
</tr>
<tr>
<td>Ongoing clinical pregnancies</td>
<td>159</td>
<td>190</td>
<td></td>
</tr>
<tr>
<td>SET</td>
<td>11/73</td>
<td>172/566</td>
<td>0.01</td>
</tr>
<tr>
<td>(15.1)</td>
<td>(30.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DET</td>
<td>122/260</td>
<td>13/83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(46.9)</td>
<td>(15.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;DET</td>
<td>26/90</td>
<td>5/17</td>
<td>NS</td>
</tr>
<tr>
<td>(28.9)</td>
<td>(29.4)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are n or n/total (%). P-values were obtained using the Chi-squared test.

SET = single-embryo transfer; DET = double-embryo transfer; >DET = more than two embryos transferred; NS = not statistically significant.

In addition, this study supports that regulated access to public funded IVF also decreases multiple pregnancies. The reduction of multiple pregnancies is the result of a judicious policy of the number of embryos to transfer. In fact, after the programme, SET increased significantly and the transfer of more than two embryos was seldom indicated. Consequently, most of the pregnancies were the result of SET during the second period, whereas only a few resulted after DET, supporting that SET is presently the most
effective strategy for reducing multiple pregnancies (ESHRE, 2001).

The cumulative pregnancy rates after the programme are in accordance with the ESHRE Campus Course for the prevention of twin pregnancies after IVF/ICSI by SET, which states that pregnancy rates higher than 30% are adequate (ESHRE, 2001).

Regarding the modalities of embryo transfer, SET resulted in a higher clinical pregnancy rate in period II compared with period I, while the opposite was seen after DET. In fact, patients selected for DET after the programme were more likely to be poor-prognosis patients since there is a direct requirement to consider transferring only one embryo and the need to justify the transfer of more than one embryo. It is also important to consider that the manner in which patients use up their three insured cycles can influence the decision to transfer or not. Under the Quebec programme, only cycles resulting in an embryo transfer count against the three insured attempts and therefore, unless a suitable embryo is available, there is always a consideration to cancel the embryo transfer.

The success of a SET policy is correlated with a good cryopreservation programme (Veleva et al., 2009). In the Quebec public IVF programme, the subsequent transfer of cryopreserved embryos is included in each cycle attempt if no pregnancy is achieved after fresh embryo transfer. In a randomized controlled trial (Thurin et al., 2004), the live birth rate after SET followed by the transfer of a single vitrified–warmed embryo was 38.8%, which is comparable to this study centre’s results in women younger than 37 years. In the same trial, the cumulative live birth after the inclusion of all vitrified–warmed cycles was 43.9%. Thus, since the Quebec public IVF programme resulted in a higher number of patients having cryopreserved embryos and a higher number of cryopreserved embryos by patient compared with period I, it is expected that the cumulative pregnancy rates after transferring all available embryos, if no live pregnancy occurred after a fresh IVF cycle, will be even higher.

In conclusion, implementation of the Quebec public IVF programme in this clinic attained the objective of decreasing multiple pregnancy rates while maintaining acceptable cumulative pregnancy rates, a more precise outcome to evaluate the impact of public IVF programmes.

Acknowledgement

M.P.V. is supported by a CIHR fellowship award.

References


Table 3 | Ongoing clinical pregnancies following the first fresh IVF cycle and multiple pregnancies according to the number of embryos transferred.

<table>
<thead>
<tr>
<th></th>
<th>Clinical pregnancies</th>
<th>Singletons</th>
<th>Twins</th>
<th>Triplets or higher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period I</td>
<td>159</td>
<td>118 (74.2)</td>
<td>39 (24.5)</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>SET</td>
<td>11 (6.9)</td>
<td>11 (100)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DET</td>
<td>122 (76.7)</td>
<td>85 (69.7)</td>
<td>35 (28.7)</td>
<td>2 (1.6)</td>
</tr>
<tr>
<td>&gt;DET</td>
<td>26 (16.4)</td>
<td>22 (84.6)</td>
<td>4 (15.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Period II</td>
<td>190</td>
<td>187 (98.4)</td>
<td>3 (1.6)</td>
<td>—</td>
</tr>
<tr>
<td>SET</td>
<td>172 (90.5)</td>
<td>171 (99.4)</td>
<td>1 (0.6)</td>
<td>—</td>
</tr>
<tr>
<td>DET</td>
<td>13 (6.8)</td>
<td>12 (92.3)</td>
<td>1 (7.7)</td>
<td>—</td>
</tr>
<tr>
<td>&gt;DET</td>
<td>5 (2.6)</td>
<td>4 (80.0)</td>
<td>1 (20.0)</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are n (%). P-values were obtained using the Chi-squared test.

SET = single-embryo transfer; DET = double-embryo transfer; >DET = more than two embryos transferred.

Table 4 | Cryopreservation: proportion of women and number of embryos available.

<table>
<thead>
<tr>
<th>Age</th>
<th>Period I</th>
<th>Period II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;37 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>105/333 (31.5)</td>
<td>303/530 (57.2)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Embryos</td>
<td>1.4 ± 2.0</td>
<td>2.6 ± 2.4</td>
<td>0.001b</td>
</tr>
<tr>
<td>≥37 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>12/166 (7.2)</td>
<td>100/285 (35.1)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Embryos</td>
<td>0.5 ± 1.8</td>
<td>1.3 ± 2.1</td>
<td>&lt;0.001b</td>
</tr>
</tbody>
</table>

Values are n (%) or mean ± SD.

*Chi-squared test

bANOVA.


Declaration: The authors report no financial or commercial conflicts of interest.

Received 31 October 2012; refereed 24 January 2013; accepted 30 January 2013.
Appendix 15


Contribution by SJ Phillips

Data analysis
Manuscript writing and editing

Citation Metrics

Google Scholar: 39
Scopus: 28
Web of Science: 22
Universal coverage of IVF pays off

M.P. Vélez1,*, M.P. Connolly2,3, I.-J. Kadoch1,4, S. Phillips4 and F. Bissonnette1,4

1Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of Montreal, Montreal, Canada
2Department of Pharmacy, Unit of PharmacoEpidemiology & PharmacoEconomics, University of Groningen, Groningen, Netherlands
3Global Market Access Solutions, St-Prex, Switzerland
4Clinique OVO, Montreal, Canada

*Correspondence address. Centre Hospitalier de l’Université de Montréal, Clinique de Procréation Assistée, 888 de Maisonneuve Blvd. East, suite 200, Montreal, QC, Canada H2L 4S8. E-mail: mdp.velez.gomez@umontreal.ca

Submitted on November 21, 2013; resubmitted on March 6, 2014; accepted on March 10, 2014

STUDY QUESTION: What was the clinical and economic impact of universal coverage of IVF in Quebec, Canada, during the first calendar year of implementation of the public IVF programme?

SUMMARY ANSWER: Universal coverage of IVF increased access to IVF treatment, decreased the multiple pregnancy rate and decreased the cost per live birth, despite increased costs per cycle.

WHAT IS KNOWN ALREADY: Public funding of IVF assures equality of access to IVF and decreases multiple pregnancies resulting from this treatment. Public IVF programmes usually mandate a predominant SET policy, the most effective approach for reducing the incidence of multiple pregnancies.

STUDY DESIGN, SIZE, DURATION: This prospective comparative cohort study involved 7364 IVF cycles performed in Quebec during 2009 and 2011 and included an economic analysis.

PARTICIPANTS/MATERIALS, SETTING, METHODS: IVF cycles performed in the five centres offering IVF treatment in Quebec during 2009, before implementation of the public IVF programme, were compared with cycles performed at the same centres during 2011, the first full calendar year following implementation of the programme. Data were obtained from the Canadian Assisted Reproductive Technologies Register (CARTR). Comparisons were made between the two periods in terms of utilization, pregnancy rates, multiple pregnancy rates and costs.

MAIN RESULTS AND THE ROLE OF CHANCE: The number of IVF cycles performed in Quebec increased by 192% after the new policy was implemented. Elective single-embryo transfer was performed in 1.6% of the cycles during Period I (2009), and increased to 31.6% during Period II (2011) (P < 0.001). Although the clinical pregnancy rate per embryo transfer was lower in 2011 than in 2009 (24.9 versus 39.9%, P < 0.001), the multiple pregnancy rate was greatly reduced (6.4 versus 29.4%, P < 0.001). The public IVF programme increased government costs per IVF treatment cycle from CAD$3730 to CAD$4759. Despite increased costs per cycle, the efficiency defined by the cost per live birth, which factored in downstream health costs up to 1 year post delivery, decreased from CAD$49 517 to CAD$43 362 per baby conceived by either fresh and frozen cycles.

LIMITATIONS, REASONS FOR CAUTION: The costs described in the economic model are likely an underestimate as they do not factor in many of the long-term costs that can occur after 1 year of age. The information collected in the Canadian ART register precludes the calculation of cumulative pregnancy rates.

WIDER IMPLICATIONS OF THE FINDINGS: Our study confirms that the implementation of a public IVF programme favouring eSET not only sharply decreases the incidence of multiple pregnancy, but also reduces the cost per live birth.

STUDY FUNDING/COMPETING INTEREST(S): M.P.V. holds a fellowship award from the Canadian Institutes of Health Research (CIHR). The economic analysis performed by M.P.C. was supported by an unrestricted grant from Ferring Pharmaceutical.

Key words: in vitro fertilization / single-embryo transfer / economics / multiple pregnancy / cost per pregnancy
Introduction

Through public funding of in vitro fertilization (IVF), governments can assure equality of access to this type of treatment while decreasing the health-care expenses associated with multiple pregnancies, the main drawback of the injudicious use of assisted reproductive technologies (ART). Public IVF programmes usually mandate a predominant single-embryo transfer (SET) policy, the most effective approach for reducing the incidence of multiple pregnancy after ART (ESHRE, 2001), and the practice of SET has been encouraged through public funding by many countries (Maheshwari et al., 2011). In Canada, the only province that universally covers the costs of IVF is Quebec (Bissonnette et al., 2011). Nonetheless, disagreement persists worldwide on the number of embryos that are safe and appropriate to transfer and on the best way to regulate this decision (Gleicher, 2011; Johnson et al., 2011; Khalaf et al., 2011).

In Canada, health care is the responsibility of the individual provinces. In the Province of Quebec, until recently, the cost of all IVF procedures was covered by the patient and partially reimbursed as a 50% tax rebate. There was no regulation regarding the number of embryos to transfer or the number of treatment cycles provided to each patient. On 5 August 2010, the Quebec government introduced a public IVF programme, marking the beginning of a new era in the field of ART in the province. The objective was to increase access to IVF and lower the cost of multiple pregnancies from ART, specifically by reducing the proportion of multiple pregnancies resulting from IVF from 30 to 5% (Gouvernement de Québec, 2012). Under this programme, all costs related to IVF are covered by Quebec’s universal health insurance plan. This includes the cost of all medical procedures related to IVF for three stimulated cycles, or up to six modified natural cycles (mnIVF). A cycle counts only when an embryo transfer has been performed. Patients who have excess embryos cryopreserved following an IVF cycle are obliged to have those embryos thawed for transfer before embarking on another ovarian stimulation cycle, but frozen/thawed embryo transfers (FET) do not count against their three attempts. The cost of gonadotrophins is reimbursed according to the individual’s public medication insurance plan, which ranges between a 68% and a 100% reimbursement rate; this medication reimbursement plan has not changed since the advent of the public IVF programme. The new policy stipulates that only one embryo may be transferred at a time. However, the law does allow for clinicians to transfer a maximum of two embryos when the woman is 36 years old, and a maximum of three embryos or two blastocysts when the woman is 37 years old or over (Gouvernement de Québec, 2010).

We previously reported the outcomes of IVF cycles in Quebec in the first 3 months of provincially funded ART (Bissonnette et al., 2011). The aim of the present study was 2-fold: to demonstrate that the sharp decrease in the multiple pregnancy rate remained constant into the second year, and to assess the economic effect of full government coverage of IVF treatment in Quebec.

Materials and Methods

Study population

Two periods were compared in our study. Period I included information from all IVF cycles performed in the five centres offering IVF treatment in Quebec during 2009, the year prior to the start of the Quebec public IVF programme. Period II comprises all IVF cycles performed in the same centres during 2011, the first full calendar year of the programme. IVF cycles involving donor oocytes or gestational carriers and mnIVF were excluded. Data were obtained from the Canadian Assisted Reproductive Technologies Register (CARTR). CARTR collects treatment cycle data from Canadian fertility centres that are using ART. Staff at each centre provide information for each IVF cycle initiated. The completely anonymous case records are sent electronically each year to the CARTR coordinating centre, where they are checked for accuracy and completeness (Gunby et al., 2011).

Stimulation protocol, oocyte retrieval and embryology procedures

As described in our previous evaluation of the first 3 months of the Quebec public IVF programme (Bissonnette et al., 2011), each centre applied its own standard protocols for ovarian stimulation and laboratory procedures. Ovarian stimulation protocols, including that of long gonadotrophin-releasing hormone (GnRH) agonist, short GnRH agonist and GnRH antagonist, were selected based on physician preference and patient characteristics. Oocyte retrieval was performed 34.5–36 h after the administration of human chorionic gonadotrophin, according to individual clinic policy. Insemination was performed using standard IVF or intracytoplasmic sperm injection. Embryo culture was performed using standardized procedures in all centres.

Embryo transfer

Embryo transfer was performed in all centres under ultrasound guidance on Day 2, Day 3, or at the blastocyst stage, depending on cycle-specific characteristics. Each clinic applied its own internal policies with respect to embryo quality and selection and regarding the pertinence of transferring more than one embryo. In general, the woman’s age, her IVF history and the quality of the embryos were primary factors in a decision to transfer multiple embryos. Embryo quality characteristics were applied when selecting suitable embryos for cryopreservation, based on the clinic’s internal protocols. Oocyte and embryo development parameters were strictly applied to eliminate embryos with very low implantation potential.

Modalities of embryo transfer

Elective single-embryo transfer (eSET) refers to the transfer of only one embryo when at least one more embryo was available for cryopreservation at the time of transfer. Non-elective single-embryo transfer (neSET) refers to the transfer of the only embryo available at the time of transfer. Elective double-embryo transfer (eDET) refers to the transfer of two embryos when more than two embryos were available at the time of transfer. Non-elective double-embryo transfer (neDET) refers to the transfer of the only two embryos available at the time of transfer. More than double-embryo transfer (≥DET) refers to the transfer of more than two embryos.

Outcomes

Pregnancy was assessed by serum human chorionic gonadotrophin concentration 15 days after oocyte retrieval (≥25 IU) and clinical pregnancy was determined by ultrasonographic evidence of an intrauterine fetal heartbeat between 7 and 8 weeks of gestation. Clinical pregnancies exclude ectopic pregnancies. Multiple pregnancies were defined according to the number of fetuses with a positive heartbeat. Frozen embryos were defined as the number of surplus embryos available for cryopreservation.
Economic analysis of the public IVF programme

A decision-analytic model was developed to assess the economic effect of the introduction of universal IVF coverage in Quebec by comparing the cost implications of the former tax-credit reimbursement system with those of the new public IVF system. The analytic framework for assessing the efficiency of policy changes differs from a cost-effectiveness analysis, which compares alternative treatment interventions among people with similar medical conditions. Policy analyses are methodologically diverse and seek to determine the policy goals and economics of different policy alternatives. Because access to care can be influenced by reimbursement, the populations considered in policy analysis may not be comparable. This is particularly relevant in the analysis described here, as access to the universal coverage of IVF in Quebec increased the demand for fertility services by nearly 200% within the first 12 months. Furthermore, complete reimbursement would likely allow access to women from a lower socioeconomic status who could not previously afford IVF treatment, a group of patients who also have an increased risk of adverse birth outcomes (Blumenshine et al., 2010).

In order to estimate the downstream costs of treatment related to multiple pregnancies, we projected live birth rates from the clinical pregnancy rates reported to CARTR by the five Quebec clinics providing IVF treatment during 2009 and 2011. Additionally, the number of children born from cryopreserved cycles was determined based on 177 and 670 frozen cycles performed in 2009 and 2011, respectively, adjusted for miscarriages. The median gonadotrophin dose reported in CARTR by the five centres providing IVF treatment in Quebec was used to estimate the ovarian stimulation costs during the years studied. All costs in the model were adjusted to 2012 to compare outcomes based on comparable costs and to be able to interpret efficiency metrics related to the reimbursement policy change. Costs were inflated by 4%, based on current health cost inflation in Canada over the past few years (Canadian Institute for Health Information, 2011).

Our model accounted for costs attributed to multiple pregnancy, preterm labour, preterm birth, neonatal admissions, antenatal care, vaginal and Cae-sarean delivery, miscarriage, cerebral palsy, neonatal care, and readmission within the first year of life. Probabilities for pregnancy-related events captured in the model are described in Table I. Cost data for each event were obtained from published references in Canada (Canadian Institute for Health Information, 2006; Chuck and Yan, 2009).

Statistical analysis

Proportion comparisons were performed by Chi-square test or Fisher exact test when indicated. The Student t-test was used to compare means. A P-value of <0.05 was considered to be statistically significant. Statistical analysis was performed using the STATA 10.0 software (Stata Corporation, College Station, TX, USA).

Because the data analysed are publicly available through the Canadian Fertility and Andrology Society website and do not include patient-specific information, this study did not require Institutional Review Board approval.

Results

A total of 1875 fresh IVF cycles were performed in Quebec during 2009 and 3489 cycles were performed during 2011, representing an increase of 192%. Characteristics of these cycles are presented in Table II. The proportion of women aged 35–39 was similar in the years studied; however, the proportion of women younger than 35 decreased in 2011, and the proportion of women aged 40 and older increased. The proportion of women experiencing their first IVF cycle was higher in 2009 than in 2011, as was the mean gonadotrophin dose. The proportion of cycles progressing to oocyte retrieval remained constant, while the proportion of cycles achieving embryo transfer decreased. The pregnancy rates were lower in 2011 compared with 2009 for all age categories. After the initiation of the programme, the multiple pregnancy rates decreased to 1/8th of its previous value in women younger than 35, and to less than half its previous value in women aged 35 and older (Table II).

As shown in Fig. 1, elective single-embryo transfer (eSET) was performed in only 1.6% of cycles during 2009, which dramatically increased

<table>
<thead>
<tr>
<th>Source</th>
<th>Probability</th>
<th>Table I Medical probabilities applied in reimbursement policy analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gunby (2010)</td>
<td>0.154</td>
<td>Miscarriage</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.007</td>
<td>Stillbirth</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.0132</td>
<td>Singleton pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.0229</td>
<td>Higher order pregnancy</td>
</tr>
<tr>
<td>Cassell et al. (2004)</td>
<td>0.046</td>
<td>Preterm labour (no delivery)</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.368</td>
<td>Twin pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.432</td>
<td>Higher order pregnancy</td>
</tr>
<tr>
<td>Canadian Institute for Health Information (2009)</td>
<td>0.07</td>
<td>Singleton pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.56</td>
<td>Twin pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.98</td>
<td>Higher order pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.00216</td>
<td>Neonatal deaths</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.009</td>
<td>Twin pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>0.038</td>
<td>Higher order pregnancy</td>
</tr>
<tr>
<td>Katalinic et al. (2004) and Pinborg et al. (2004)</td>
<td>0.335</td>
<td>Single pregnancy</td>
</tr>
<tr>
<td>Katalinic et al. (2004) and Pinborg et al. (2004)</td>
<td>0.6135</td>
<td>Twin pregnancy</td>
</tr>
<tr>
<td>Gunby (2010)</td>
<td>1b</td>
<td>High order pregnancy</td>
</tr>
<tr>
<td>Martens et al. (2004)</td>
<td>0.0654</td>
<td>Re-hospitalization based on premature &lt;37 weeks</td>
</tr>
<tr>
<td>Public Health Agency of Canada (2008)</td>
<td>0.031</td>
<td>Maternal re-hospitalization C-section</td>
</tr>
<tr>
<td>Public Health Agency of Canada (2008)</td>
<td>0.018</td>
<td>Maternal re-hospitalization vaginal delivery</td>
</tr>
</tbody>
</table>

*Based on average C-section rates reported by Pinborg et al. (2004) and Katalinic et al. (2004).

*Based on Canadian expert opinion.
to 31.6% in 2011 ($P < 0.001$). The proportions of elective double-embryo transfer (eDET) and the transfer of more than two embryos (>DET) revealed a sharp decrease in 2011 ($P < 0.001$).

Table III shows the outcomes of frozen/thawed embryo transfer cycles performed in Quebec preceding and following universal IVF coverage. Overall, the proportion of cycles having embryos frozen was similar in both periods; however by age group, it was higher in the two younger age groups during 2011. The mean number of frozen embryos per patient was higher in 2009 compared with 2011, and this applied to all age groups.

In 2009, 177 FET created from fresh cycles were performed, compared with 670 in 2011. The proportion of cycles performed was similar for all age categories. Although the mean number of frozen/thawed embryos transferred in 2009 was higher than in 2011, the clinical pregnancy rate and the multiple pregnancy rate were similar in both study years.

### Economics

The costs to the Quebec government associated with funding IVF increased substantially in the first years of the public IVF programme from CAD$7 million to CAD$26 million (Table IV). The comparative total cost, which factored in antenatal, birth and complication costs for IVF pregnancies, was CAD$31.4 million for the former partial reimbursement by tax-credit and CAD$49.1 million for universal IVF coverage.

The public IVF programme increased government costs per IVF treatment cycle from CAD $3730 to CAD$4759. The health system efficiency per cycle, which considered all downstream care-related costs, decreased with universal IVF coverage to CAD$8960 per cycle from CAD$16 747 per cycle in 2009. Based on the number of projected children born from 2009 fresh and frozen cycles and the projected live births from the 2011 cohort, the cost per baby surviving to 1 year of age decreased from CAD$49 517 to CAD$43 362 per live birth with universal reimbursement.

### Discussion

The Quebec public IVF programme immediately attained the objectives of increasing access to IVF and reducing multiple pregnancy rates. There was a decrease in the proportion of patients having embryo transfer in 2011. In fact, the manner in which patients use up their three insured

---

**Table II Outcomes of fresh IVF cycles performed in Quebec preceding and following universal IVF coverage.**

<table>
<thead>
<tr>
<th></th>
<th>2009</th>
<th>2011</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cycles</td>
<td>1875</td>
<td>5489</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>854  (45.5)</td>
<td>2032 (37.0)</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>673  (35.9)</td>
<td>1996 (36.4)</td>
<td></td>
</tr>
<tr>
<td>≥ 40 years</td>
<td>348  (18.6)</td>
<td>1461 (26.6)</td>
<td></td>
</tr>
<tr>
<td>Number of previous IVF cycles*</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>None</td>
<td>944  (73.0)</td>
<td>3403 (62.0)</td>
<td></td>
</tr>
<tr>
<td>One</td>
<td>259  (20.0)</td>
<td>1317 (24.0)</td>
<td></td>
</tr>
<tr>
<td>Two or more</td>
<td>91   (7.0)</td>
<td>789 (14.0)</td>
<td></td>
</tr>
<tr>
<td>Gonadotrophin dose IU (mean, SD)</td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>Oocyte retrievals</td>
<td>1743 (93.0)</td>
<td>5110 (93.1)</td>
<td>0.8</td>
</tr>
<tr>
<td>Embryo transfers</td>
<td>1660 (88.5)</td>
<td>4361 (79.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Clinical pregnancies per embryo transfer</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>372/778 (47.8)</td>
<td>566/1677 (33.8)</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>239/607 (39.4)</td>
<td>384/1586 (24.2)</td>
<td></td>
</tr>
<tr>
<td>≥ 40 years</td>
<td>52/275 (18.9)</td>
<td>134/1098 (12.2)</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancies per clinical pregnancy</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>124 (33.3)</td>
<td>22 (3.9)</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>60 (25.1)</td>
<td>35 (9.1)</td>
<td></td>
</tr>
<tr>
<td>≥ 40 years</td>
<td>11 (21.2)</td>
<td>12 (9.0)</td>
<td></td>
</tr>
</tbody>
</table>

Values are n (%), unless otherwise stated.

*Missing information in 31% of the cycles for 2009.

**Figure 1** Proportion of cycles using different modalities of embryo transfer, in period I (2009) and period II (2011).
cycles can influence the decision whether to transfer or not. Under the Quebec public IVF programme, only cycles resulting in an embryo transfer count against the three insured attempts, and therefore, unless a suitable embryo is available, there is always the option of cancelling the embryo transfer.

The lower proportion of women with previous IVF cycles among the women receiving treatment in 2009 compared with those receiving the same treatment in 2011 might demonstrate existing barriers in access to women receiving treatment in 2009 compared with those receiving the embryo transfer.

It is not possible to determine whether the lower proportion of women with previous IVF cycles among the women receiving treatment in 2009 compared with those receiving the same treatment in 2011 might demonstrate existing barriers in access to women receiving treatment in 2009 compared with those receiving the embryo transfer.

The increase in access to IVF after implementing the public programme was mainly achieved through the impact of the elimination of multiple pregnancies per clinical pregnancy. However, as also a consequence of the new SET policy, the rate of embryo cryopreservation was higher in the second period (although this only applied to women under 40), thus permitting more cycles to be undertaken.

Although there were fewer embryos available for cryopreservation per patient during the second period, which could be explained by a tendency among physicians towards milder ovarian stimulation in recent years. There is a 2-year difference in our study; therefore treating physicians may be stimulating their patients less aggressively than before, especially when only one embryo is transferred, as is suggested by the lower gonadotrophin dose prescribed during 2011 compared with 2009. There was no significant difference in the pregnancy rate per FET, although the mean number of frozen/thawed embryos transferred in 2011 was lower than in 2009. We hypothesize that because only one embryo is transferred in most patients under the new policy, there may be one or more other embryos of equal quality that will be frozen; previously, these better-quality embryos would also have been transferred, leaving only the poorer-quality embryos for freezing. At the present time, no information is available on embryo quality to test this hypothesis.

The number of clinics offering IVF treatment, their relative proximity and their opening hours, factors that, along with the cost of treatment, are the main determinants of IVF utilization/access (Collins, 2002), had been the same before the advent of the public funding.

Because the use of eSET was much greater in the second period, a decrease in the pregnancy rate was seen, especially in younger women.

### Table III: Outcomes of frozen/thawed embryo transfer cycles performed in Quebec preceding and following universal IVF coverage.

<table>
<thead>
<tr>
<th></th>
<th>2009</th>
<th>2011</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of women with embryos available for cryopreservation (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>47.8</td>
<td>54.0</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>28.5</td>
<td>32.7</td>
<td></td>
</tr>
<tr>
<td>≥40 years</td>
<td>12.6</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>Frozen embryos per patient, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>4.70 (3.1)</td>
<td>3.54 (2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>35–39 years</td>
<td>4.16 (2.6)</td>
<td>3.10 (2.7)</td>
<td></td>
</tr>
<tr>
<td>≥40 years</td>
<td>4.70 (3.7)</td>
<td>2.99 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Frozen/thawed embryo transfer cycles (FET), n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>103 (58.2)</td>
<td>393 (58.7)</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>55 (31.1)</td>
<td>216 (32.2)</td>
<td></td>
</tr>
<tr>
<td>≥40 years</td>
<td>19 (10.7)</td>
<td>61 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Number of frozen/thawed embryos transferred, mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical pregnancy rate per FET, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>26 (25.2)</td>
<td>84 (21.4)</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>18 (32.7)</td>
<td>39 (18.1)</td>
<td></td>
</tr>
<tr>
<td>≥40 years</td>
<td>1 (5.3)</td>
<td>7 (11.5)</td>
<td></td>
</tr>
<tr>
<td>Multiple pregnancies per clinical pregnancy, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years</td>
<td>4 (15.4)</td>
<td>12 (14.3)</td>
<td></td>
</tr>
<tr>
<td>35–39 years</td>
<td>3 (17.7)</td>
<td>3 (12.8)</td>
<td></td>
</tr>
<tr>
<td>≥40 years</td>
<td>0</td>
<td>2 (28.6)</td>
<td></td>
</tr>
</tbody>
</table>

*Embryos created from fresh cycles performed during the same calendar year.

### Table IV: Costs to the Quebec government of implementing universal IVF coverage and changes in efficiency based on differences in outcomes achieved for the two policies.

<table>
<thead>
<tr>
<th></th>
<th>50% tax rebate policy 2009</th>
<th>Universal IVF policy 2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of fresh IVF cycles</td>
<td>1875</td>
<td>5489</td>
</tr>
<tr>
<td>Number of FET</td>
<td>177</td>
<td>670</td>
</tr>
<tr>
<td>Sum of all costs</td>
<td>C$31 402 410</td>
<td>C$49 184 098</td>
</tr>
<tr>
<td>Total costs of IVF treatment</td>
<td>C$6 995 164</td>
<td>C$26 120 797</td>
</tr>
<tr>
<td>IVF pregnancy medical costs</td>
<td>C$24 407 246</td>
<td>C$23 063 301</td>
</tr>
<tr>
<td>Pregnancy costs, miscarriage, preterm labour</td>
<td>C$1 691 941</td>
<td>C$2 056 421</td>
</tr>
<tr>
<td>Hospitalization costs including NICU</td>
<td>C$15 699 392</td>
<td>C$15 775 241</td>
</tr>
<tr>
<td>First year costs and CP cases</td>
<td>C$7 015 913</td>
<td>C$5 231 639</td>
</tr>
<tr>
<td>Total cost per cycle</td>
<td>C$16 747</td>
<td>C$8960</td>
</tr>
<tr>
<td>IVF treatment costs per cycle</td>
<td>C$3730</td>
<td>C$4759</td>
</tr>
<tr>
<td>Medical costs per cycle&lt;sup&gt;a&lt;/sup&gt;</td>
<td>C$13 017</td>
<td>C$4201</td>
</tr>
<tr>
<td>Cost per bab&lt;sup&gt;b&lt;/sup&gt;</td>
<td>C$49 517</td>
<td>C$43 362</td>
</tr>
</tbody>
</table>

<sup>a</sup>Includes all downstream related maternity and child costs to 1 year.
<sup>b</sup>Sum of all costs per cycle/1 year survivors.
Sundstrom, 2005; Van Landuyt et al., 2006). This is the result of a judicious policy regarding the number of embryos to transfer. In our case, single-embryo transfer increased significantly after the programme was instituted, and more than two embryos were seldom transferred, supporting the hypothesis that single-embryo transfer is presently the most effective strategy towards reducing multiple pregnancies (ESHRE, 2001).

Over the past 10 years, conventional wisdom in fertility policy has held that the public funding of IVF will finance itself due to the reduction of multiple pregnancies and their high cost to the health system. This fails to account for the prevailing unmet need for IVF treatment that often exists in society and the increase in demand that may occur following such changes in funding. In Quebec, the demand for IVF treatment increased by nearly 200% following the inclusion of the treatment in the universal coverage system. This brought a dramatic increase in cost to the government, as noted above, with the cost per IVF cycle increasing from CAD$3730 to CAD $4759. Although savings were achieved in terms of reduced neonatal care, re-hospitalization and complications, there were also increases in antenatal and delivery costs due to more children being conceived and born by means of IVF treatment.

A better measure of a policy’s success can be assessed by estimating efficiency outputs which rationalize costs in relation to the outcomes achieved in alternative policy scenarios. In Quebec, the introduction of universal IVF coverage increased the number of clinical pregnancies and projected live births. The reduced medical cost per cycle and reduced cost per live birth indicate that savings are being achieved with the new public IVF programme. Despite the surge in demand due to the newly available possibility for many to fulfill a previously unmet need, and despite the increased cost of IVF treatment compared with the former tax-credit reimbursement system, the efficiency of the policy to reduce cost per baby has been achieved. The costs described here are likely an underestimate, as they do not factor in many of the long-term costs that can arise from complications. Furthermore, as the emphasis is exclusively on health-care costs, our analysis fails to take into account the long-term contribution that children offer to society, and their contribution to fiscal sustainability (Connolly et al., 2010).

Increasingly, modelling is used to bridge gaps in available evidence to be able to understand the long-term implications of policy changes and medical treatment practices. In our study, the collection of longitudinal data on patients was not possible due to the privacy and ethical concerns that have prevented the establishment of a central register that reports the outcomes of IVF treatments. Moreover, collecting such information was not feasible in this study, as the cost of collecting primary data related to the recent policy change would have been prohibitively high. In such instances, modelling can be an informative bridge to establish links between ongoing pregnancies and their likely outcomes based on previously reported probability outcomes in comparable populations. To reflect the increased risks associated with IVF-conceived children, we considered probabilities and rates reported in studies conducted in other ART populations. However, in some instances, it was not possible to find data specific to ART-conceived children, and therefore, the cost estimates of our analysis should be viewed as conservative.

It should be stressed that the implementation of a public IVF programme can only be adequately assessed by means of a comprehensive health-surveillance tool. The Canadian ART register was established for the collection of treatment cycle data from fertility centres using ART and is financially supported by voluntary participating ART centres (Gunby, 2012). Although this register addresses important aspects of IVF, the information needs to be expanded and integrated into a comprehensive health-care monitoring system that includes the follow-up of women treated by ART, and the health of children born after these treatments.

Acknowledgements

The authors acknowledge the effort of the clinical, laboratory and administrative personnel of the IVF centres that initially implemented the Quebec public IVF programme: McGill University Reproductive Centre, the Montreal Fertility Clinic, the OVO Fertility Clinic, and Procrea Fertility. Many thanks to Joanne Gunby, CARTR co-ordinator, who provided the clinical data from CARTR and critically revised the article for content.

Authors’ roles

M.P.V., M.P.C. and F.B. were all involved in the conception and design of the study. M.P.V. carried out analysis and interpretation of clinical data, in addition to drafting the manuscript. M.P.C. performed the economic analysis and carried out data interpretation, as well as a critical review of the article for content. S.P., L.-J.K. and F.B. carried out data interpretation and also commented on and revised the article for content.

Funding

M.P.V. holds a fellowship award from the Canadian Institutes of Health Research (CIHR). The economic analysis performed by M.P.C. was supported by an unrestricted grant from Ferring Pharmaceuticals.

Conflict of interest

None declared.

References


Appendix 16


Contribution by SJ Phillips

Concept
Data collection
Data analysis
Manuscript writing and editing

Citation Metrics (book chapter)

Google Scholar: 0
Scopus: 0
Chapter 22
The Quebec Experience—One Plus One Equals Two at Once: Presenting Cumulative Pregnancy Rates as the Ideal Outcome in Elective SET Programmes

Maria P. Vélez, Isaac-Jacques Kadoch, Simon J. Phillips, and Francois Bissonnette

Introduction

Single Embryo Transfer (SET) is the most effective approach to reduce the incidence of multiple pregnancies associated with Assisted Reproductive Technologies [1]. Although the pregnancy rate after one fresh SET is reported to be lower compared with one fresh double embryo transfer (DET), no difference exists when one DET is compared with elective SET followed by one Frozen embryo Transfer (FET) [2]. Elective SET requires the selection of good prognosis patients and the transfer of the best high quality embryo [3]. As noted elsewhere in this volume, sophisticated molecular techniques continue to be refined for evaluation of the chromosomal competencies of embryos, thus permitting an improved selection process to enable SET. Of note, several observational studies comparing elective SET with DET have not found differences in terms of pregnancy rate among both groups [3–5]. The few Randomized Controlled Trials that have compared a single cycle of DET with one cycle of fresh SET followed by one frozen/thawed SET have shown that there is no significant difference in terms of cumulative live birth rates [2]. Moreover, in regard to health expenses, elective SET embryo is substantially cheaper than DET in women younger than 38 years with a good prognosis [5].
Public financing of assisted reproductive technologies (ART) is intended to increase access to fertility treatments by reducing the financial burden to patients. In counterpart, governments aim to reduce the health expenses associated with multiple pregnancies attributable to the use of ART. Although comprehensive government initiatives are associated with greater utilization of fertility treatments and lower rates of multiple pregnancies [6, 7], critics of public financing argue that public programmes are also associated with lower pregnancy rates [8].

In Canada, health care is the responsibility of the individual provinces. In the Province of Quebec, the cost of all IVF procedures was covered by the patient and partially reimbursed as a 50 % tax rebate before 2010. There was no regulation regarding the number of embryos to transfer or the number of treatment cycles provided to each patient. On 5 August 2010, the Quebec government introduced a public IVF programme, marking the beginning of a new era in the field of ART here. Under this programme, all costs related to IVF are covered by Quebec’s universal health insurance plan. This includes the cost of all medical procedures related to IVF for three stimulated cycles, or up to six modified natural cycles (mnIVF) [9, 10].

We previously assessed the clinical outcomes and the economic effect of the IVF cycles performed in Quebec during the first year of provincially funded ART [6, 7]. We reported that the implementation of a public IVF programme favouring elective SET not only sharply decreases the incidence of multiple pregnancy but also reduces the cost per live birth [7]. In addition, we have shown that the cumulative pregnancy rate per initiated cycle (i.e. the proportion of clinical pregnancies after the first fresh IVF cycle, including the resulting first frozen/thawed embryo transfer) was comparable to DET before the public IVF programme in one of the IVF centres offering ART treatment in Quebec [11]. In this chapter, we aim to demonstrate that the pregnancy rate after one fresh elective SET plus one FET is comparable to the pregnancy rate after elective DET using data from the whole province of Quebec. We underscore the importance of presenting cumulative pregnancy rates as the preferred outcome to evaluate the impact of elective SET programmes.

Methods

Study Population

The methodology of this prospective comparative analysis has been previously described [7]. For this specific analysis, period I includes the elective DET performed in the five centres offering IVF treatment in Quebec during 2009, the year prior to the start of the Quebec public IVF programme. Period II comprises the elective SET performed in the same centres during 2011, the first full calendar year of the programme, plus the first FET from embryos created from fresh 2011 cycles. Elective Single Embryo Transfer (eSET) refers to the transfer of only one embryo when at least one more embryo was available for cryopreservation at the time of
transfer. Elective Double Embryo Transfer (eDET) refers to the transfer of two embryos when more than two embryos were available at the time of transfer.

Data were obtained from the Canadian Assisted Reproductive Technologies Register (CARTR). CARTR collects treatment cycle data from Canadian fertility centres that are using ART. Staff at each centre provides information for each IVF cycle initiated. The complete anonymous case records are sent electronically each year to the CARTR coordinating centre, where they are checked for accuracy and completeness [12].

Clinical and Laboratory Procedures

Ovarian stimulation protocols, including long gonadotropin-releasing hormone (GnRH) agonist, short GnRH agonist, and GnRH antagonist, were selected based on physician preference and patient characteristics. Oocyte retrieval was performed 36 h after the administration of human chorionic gonadotropin. Insemination was performed using standard IVF or intracytoplasmic sperm injection when indicated. Embryo culture was performed using standardized procedures. Embryo transfer was uniformly performed under ultrasound guidance on Day 2, Day 3, or at the blastocyst stage, depending on cycle-specific characteristics. Each clinic applied its own internal policies with respect to embryo quality and selection and regarding the pertinence of transferring more than one embryo. In general, the woman’s age, her IVF history, and the quality of the embryos were primary factors in a decision to transfer multiple embryos. Embryo quality characteristics were applied when selecting suitable embryos for cryopreservation, based on the clinic’s internal protocols. Oocyte and embryo development parameters were strictly applied to eliminate embryos with very low implantation potential.

Outcomes

Pregnancy was assessed by serum human chorionic gonadotropin concentration 15 days after egg retrieval (≥25 IU); and clinical pregnancy was determined by ultrasonographic evidence of intrauterine fetal heartbeat between 7 and 8 weeks of gestation. For this tabulation, clinical pregnancies excluded ectopic pregnancies. Multiple gestation was defined according to the number of embryos with positive cardiac action. Frozen embryos were defined as the number of surplus (non-transferred) embryos available for cryopreservation.

We estimated the time-limited cumulative pregnancy rate among patients undergoing a fresh eSET during 2011, plus the resulting first FET performed during the same study period. The 2011 cumulative pregnancy rate was then compared with the pregnancy rate after eDET performed in 2009. Time-limited analyses using proportions provide information on the likelihood of pregnancy per woman with a predetermined number of IVF cycles in the time period defined. This method is an
alternative to life table analysis, which although frequently used may overestimate treatment effect [13]. We then conducted a sensitivity analysis to extrapolate the cumulative pregnancy rate for those women who following a negative pregnancy test, and having cryopreserved embryos, were unable to have their first FET during 2011.

**Statistical Analysis**

Proportion comparisons were performed by chi-squared test or Fisher’s Exact test, as appropriate. Student’s t-test and ANOVA were used to compare means. A p-value <0.05 was considered to be statistically significant. Statistical analysis was performed using STATA version 10.0 (Stata Corporation, College Station, TX, USA). Because the data analysed for this investigation are publicly available through the Canadian Fertility and Andrology Society website and do not include patient-specific information, this study did not require Institutional Review Board approval.

**Results**

The mean age was similar in both groups [32.54 years (SD 3.69) in the eDET group versus 32.52 (SD 3.62) in the eSET group, \( p = 0.92 \)]. Additional characteristics are presented in Table 22.1. The two groups were similar in terms of the distribution of age categories, number of prior pregnancies, and number of previous IVF cycles. There are some differences in the diagnosis of infertility, with some indication that the eSET group is at a disadvantage for some prognostic factors. As Table 22.2 reflects, the mean number of oocytes retrieved, cleaved embryos, and embryos cryopreserved was lower in the eSET group compared with the eDET (2009).

Table 22.3 presents the outcomes of IVF cycles performed during both study periods according to the two modalities of embryo transfer. There were 514 eDET during 2009. The eDET pregnancy rate was 47.1 % in 2009, and the multiple pregnancy rate was 35.1 %. There were 1,375 eSET cycles in 2011, of which 466 (33.9 %) resulted in a pregnancy, leaving 909 women to have transfer of cryopreserved embryos (by definition, all women having eSET have at least one embryo for cryopreservation). Of these, 378 women had a first FET during 2011 with a pregnancy rate of 21.2 %, which leads to a clinical pregnancy after one fresh eSET plus one FET of 39.7 % (Table 22.3).

Next, we conducted a sensitivity analysis to extrapolate the cumulative pregnancy rate. Applying a conservative survival rate of 90 % to the remaining cryopreserved embryos, 478 women still waiting for their first FET after eSET will be able to undergo an FET. Extrapolating the 2011 FET pregnancy rate of 21.2 % to the remaining women, 101 more pregnancies would be expected. So the pregnancy rate after one fresh eSET plus one FET would be 47.1 % (647/1,375). This is equal to the 2009 eDET pregnancy rate, showing equivalence between the two approaches (\( p = 0.8 \)).
### Table 22.1  Characteristics of the Quebec study population

<table>
<thead>
<tr>
<th>Age</th>
<th>2009 (eDET), n = 514</th>
<th>2011 (eSET), n = 1,375</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;35</td>
<td>367</td>
<td>943</td>
<td>0.15</td>
</tr>
<tr>
<td>35–39</td>
<td>132</td>
<td>405</td>
<td>0.99</td>
</tr>
<tr>
<td>≥40</td>
<td>15</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>Prior pregnancy</td>
<td>0.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>266</td>
<td>620</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>134</td>
<td>313</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>114</td>
<td>442</td>
<td></td>
</tr>
<tr>
<td>Prior ART cycles</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>317</td>
<td>1,053</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>59</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>≥3</td>
<td>6</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>118</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Diagnosis category</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unexplained</td>
<td>90</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td>Male factor only</td>
<td>224</td>
<td>544</td>
<td></td>
</tr>
<tr>
<td>&gt;1 female factor</td>
<td>12</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Male + female factor</td>
<td>71</td>
<td>133</td>
<td></td>
</tr>
<tr>
<td>Endometriosis only</td>
<td>37</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Tubal only</td>
<td>41</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Ovulatory only</td>
<td>27</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>DOR only</td>
<td>6</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Other female only</td>
<td>4</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Missing</td>
<td>2</td>
<td>31</td>
<td></td>
</tr>
</tbody>
</table>

### Table 22.2  IVF outcomes in Quebec, as a function of ET strategy

<table>
<thead>
<tr>
<th>Oocytes retrieved</th>
<th>2009 (eDET), n = 514</th>
<th>Mean</th>
<th>SD</th>
<th>2011 (eSET), n = 1,375</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14.7</td>
<td>6.5</td>
<td></td>
<td>13.3</td>
<td>6.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cleaved embryos</td>
<td></td>
<td>8.9</td>
<td>4.1</td>
<td></td>
<td>7.8</td>
<td>4.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Embryos cryopreserved</td>
<td></td>
<td>4.2</td>
<td>2.9</td>
<td></td>
<td>3.18</td>
<td>2.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### Table 22.3  Clinical pregnancies following elective DET (2009) vs. elective SET + one FET (2011) in Quebec

<table>
<thead>
<tr>
<th>Transfers</th>
<th>2009 Fresh eDET cycles</th>
<th>2011 Fresh eSET cycles</th>
<th>First FET after eSET</th>
<th>Cumulative</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical pregnancies</td>
<td>242 (47.1)</td>
<td>466 (33.9)</td>
<td>80 (21.2)</td>
<td>546/1,375 (39.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>Single pregnancies</td>
<td>157 (64.9)</td>
<td>458 (98.3)</td>
<td>70 (87.5)</td>
<td>528 (96.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multiple pregnancies</td>
<td>85 (35.1)</td>
<td>8 (1.7)</td>
<td>10 (12.5)</td>
<td>18 (3.3)</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** All data presented as n (%)
Conclusion

Our goal was to present data regarding the implementation of a predominant eSET treatment strategy through public funding in the province of Quebec. One concept that was alluded to in the presentation of the initial data from this programme was that the transfer of two embryos, one at a time, would result in pregnancy rates similar to those seen when eDET was used prior to the programme [6]. This concept was recently supported with data from one of the five centres offering IVF treatment in Quebec [11]. The present analysis, which includes data from the entire Quebec province, goes towards the same direction. The extrapolated pregnancy rate after one fresh eSET plus one first FET in 2011 was not different from that of fresh eDET alone in 2009. Moreover, it is similar to the pregnancy rate of 47.7% reported in a large randomized controlled trial comparing these two treatment strategies [14]. We recognize the limitations of extrapolating clinical pregnancies, but this is our best estimate since the Canadian ART Register collects data per individual cycle, not longitudinally per patient. We consider, however, that our results are valid and conservative. Indeed, some patients who won’t be pregnant after their first FET will have cryopreserved embryos still available, increasing even more the probability of pregnancy. Our data support, therefore, that cumulative pregnancy rates should be presented as the ideal outcome in the evaluation of the effectiveness of eSET programmes.

Conflict of Interest  The authors declare no conflict.

References


Appendix 17


Contribution by SJ Phillips

- Concept
- Data collection
- Data analysis
- Manuscript writing and editing

Citation Metrics

- Google Scholar: 0
- Scopus: 0
Impact of government health coverage for ART: the results of a 5-year experience in Quebec

F. Bissonnette\textsuperscript{a,b}, S. Phillips\textsuperscript{a,b,*}, J. Sampalis\textsuperscript{c}, E.M. Dahdouh\textsuperscript{b,d}, P. St-Michel\textsuperscript{e}, W. Bucket\textsuperscript{c,f}, I.J. Kadoch\textsuperscript{a,b}, N. Mahutte\textsuperscript{g}

\textsuperscript{a}Clinique OVO, Montreal, Quebec, Canada; \textsuperscript{b}University of Montreal, Montreal, Quebec, Canada; \textsuperscript{c}McGill University, Montreal, Quebec, Canada; \textsuperscript{d}ART Center, CHU Sainte-Justine, Montreal, Quebec, Canada; \textsuperscript{e}Procrea Clinics, Quebec, Canada; \textsuperscript{f}MUHC Reproductive Centre, Montreal, Quebec, Canada; \textsuperscript{g}The Montreal Fertility Centre, Montreal, Quebec, Canada

\textsuperscript{*}Corresponding author. E-mail address: s.phillips@cliniqueovo.com (S. Phillips).
Abstract An analysis of national registry data for 5 years of in-vitro fertilization (IVF) funding in Quebec, Canada was compared with the previous complete year of non-funded IVF cycles, as well as the first complete year following the end of funding. The number of cycles, livebirth rates, age group of patients treated, use of donor gametes, multiple pregnancy rates and cycle cancellation rates were assessed. The total number of IVF cycles performed increased dramatically during the funded period, averaging over 10,000 cycles per year. There was no change in the age group distribution of patients treated, but less egg donation was performed. Interestingly, funding was also associated with an increase in the IVF cycle cancellation rate (17.0% versus 34.4%, \( P < 0.001 \)), a dramatic decline in the multiple pregnancy rate (25.6% versus 4.9%, \( P < 0.001 \)), and a decline in the livebirth rate per fresh embryo transfer in stimulated IVF cycles (32.3% versus 25.5%, \( P < 0.001 \)). Although the livebirth rate for stimulated IVF declined, over 9000 babies were born as a result of the coverage. Lessons learned from this experience could help develop a more fiscally responsible programme that still facilitates access to IVF care.

KEYWORDS: IVF, public funding, multiple pregnancy rates
<A>Introduction</A>

In August 2010, the Provincial Government of Quebec introduced public coverage for assisted reproductive technology (ART) treatment (Gazette Officielle du Québec, 2010). In parallel, the number of embryos that could be transferred was controlled. The law encouraged single embryo transfer (SET) in all cases, but permitted up to two embryos in women aged $\leq 36$ years and up to three embryos in women aged $\geq 37$ years. The results of the first few months of the programme demonstrated a dramatic decrease in the multiple pregnancy rate due to the increased use of elective SET (Bissonnette et al., 2011). The law covered ART treatment for all residents of Quebec via the provincial health plan. There were no exclusion criteria in terms of age, previous history of tubal ligation/vasectomy, previous pregnancies, sexuality or marital status. The coverage provided for three stimulated in-vitro fertilization (IVF) cycles including medication, along with any associated procedures such as surgical sperm retrieval or donor semen, and the frozen embryo transfers (FET) resultant from those egg retrievals. Any frozen embryos had to be used before further ovarian stimulation could be undertaken. Egg donation was covered if the egg donor herself was a holder of a valid Quebec health card, but obtaining donor eggs from out of province or from an egg bank was not covered.

An IVF cycle only counted towards the three attempts if an embryo transfer occurred. Therefore, any cycles cancelled prior to embryo transfer (poor ovarian stimulation, no eggs at retrieval, failed fertilization or no high-quality embryos available for transfer) were not counted. Furthermore, a successful live birth reset the counter.

After a little more than 5 years of operation, on 15 November 2015, the programme was terminated (Gazette Officielle du Québec, 2015). A new law
removed the coverage of IVF, except in the case of fertility preservation for oncologic reasons, although only ovarian stimulation, oocyte retrieval and cryopreservation remained covered; fertilization by intracytoplasmic sperm injection, embryo culture and future use of the cryopreserved materiel were not included. Furthermore, the law increased restrictions on embryo transfer, mandating SET for all women aged <37 years, while permitting two embryos to be transferred in patients aged ≥37 if written justification was provided in the patient file. In sharp contrast to IVF, intrauterine insemination (IUI) was covered to a maximum of nine attempts, including medication, monitoring, semen preparation and insemination. The purchase of donor semen for IUI was no longer covered.

Materials and methods
In Canada, professionals working in the field of assisted human reproduction meet under the auspices of the Canadian Fertility and Andrology Society (CFAS). Since 2000, CFAS has managed a registry of ART cycles performed across the country [Canadian Assisted Reproductive Technology Registry (CARTR)]. Since 2014, CARTR has been managed by the Better Outcomes Registry Network (BORN) Ontario. BORN is the province of Ontario’s registry for births within the province. Although based in one province, for the ART registry, BORN manages the data from all IVF clinics across Canada. BORN does not receive any patient identifying information from provinces other than Ontario, and is managed by very strict privacy regulations.

Data from CARTR-BORN is available upon request for research purposes. There was no direct patient involvement in this study and data were already anonymized: as such, no ethical approval was required according to Canadian
Institutes of Health Research policy on ethical conduct for research involving humans. This study used CARTR-BORN data from August 2010 until November 2015, representing the period when ART was covered under Quebec provincial health insurance, and from 2009 and 2016 to compare data from years either side of the coverage when ART was within the private domain.

Data are reported as livebirth rates per embryo transfer. During the 64 months of the programme, in addition to the live births that were reported to clinics, there were an additional 1310 clinical pregnancies for which the livebirth data were not reported by patients. As these pregnancies were confirmed by ultrasound at the clinics, a 15% loss of pregnancy (Avalos et al., 2012) was assumed, and the remaining 85% of these lost-to-follow-up clinical pregnancies were added to the livebirth data. There were 27 similar cases in 2009 and 124 cases in 2016, of which 85% were also included in the livebirth data.

Statistical analysis was performed using Chi-squared test, and the result was considered to be significant when \( P<0.05 \). No funding was obtained for this study.

Ethical approval was not required for this study according to the Canadian Tri-council Policy Statement on Ethical Conduct for Research Involving Humans, as data were obtained from a centralized anonymized databank.

**Results**

The total number of cycles performed per year increased dramatically once the programme became established (see Figure 1). Interestingly, however, the proportion of patients treated by age was not affected by the availability of insured IVF treatments (see Figure 2). The number of fresh IVF cycles started decreased dramatically in 2016, while the number of frozen–thaw cycles started remained
relatively stable in 2016. It is important to note that upon termination of the programme in November 2015, all patients with cryopreserved embryos in storage from the programme still had coverage for their FET cycles until a pregnancy was obtained. In addition, all patients with a valid prescription for IVF obtained prior to 15 November 2015 were also covered for their fresh IVF attempt and all FET cycles as a result of that attempt. Thus, despite the change in the law, some fresh and frozen–thaw cycles performed in 2016 remained covered.

<insert Figures 1 and 2 near here>

The overall cancellation rate for fresh IVF cycles increased from 17% in 2009/2016 (combined) to 34.4% during the programme ($P<0.001$) (Table 1).

<insert Table 1 near here>

<B>Use of donor gametes

During the funded period, the percentage of IVF cycles that were combined with donor sperm decreased (8.2% versus 6.2%, $P<0.001$). The use of donor eggs in IVF also declined significantly during the insured period (4.5% versus 2.4%, $P<0.001$), and this reduction was seen for all age groups.

<B>Livebirth rates

The overall livebirth rate per fresh embryo transfer decreased during the funded period compared with 2009/2016 (33.9% versus 23.7% between 2010 and 2015, $P<0.001$) (Table 2). Although significant for both fresh and frozen IVF cycles, the
absolute percentage point decline was greater in fresh cycles. The complete results for stimulated cycles, natural modified cycles and FET cycles broken down by age are presented in Tables 3–5. The reduction in the livebirth rate per transfer for fresh embryo transfer following IVF with ovarian stimulation was significant in all age groups except for 39–40 years (Table 3).

<insert Tables 2–5 near here>

There were 9232 live births between 2010 and 2015, and although the multiple pregnancy rate was reduced significantly by the programme, as reported previously (Bissonnette et al., 2011), a small proportion of these live births involved the birth of more than one baby. Overall, more than 9232 babies were born as a result of the programme.

<B>Use of single embryo transfer

As should be expected from a programme twinned with legislation controlling the number of embryos to be transferred, the use of SET in stimulated IVF cycles and FET cycles increased significantly (Table 6) when comparing 2009 with 2010–2015 (IVF 9.2% versus 64.3%, \( P<0.0001 \); FET 10.0% versus 73.5%, \( P<0.0001 \)). Moreover, because the change in coverage in 2015 coincided with even tighter restrictions on the number of embryos that could be transferred, the use of SET increased further in 2016 (IVF 71.5%, FET 86.7%). Not surprisingly, there was no difference in the use of SET in the natural cycle IVF groups. Coincident with increased use of SET, the multiple pregnancy rate decreased from 25.6% in 2009 to
3.3–7% during the years of the programme, and was 4.5% in 2016 during the return to privately funded cycles (Table 7).

<insert Tables 6 and 7 near here>

Discussion
There was an enormous increase in the number of cycles performed during the insured period, with total cycle numbers reaching close to 12,000 in 2013, and averaging over 10,000 per year. This represented a five-fold increase compared with the number of cycles performed in 2009, despite the fact that the proportion of patients in each age group did not change. However, this coincided with a reduction in the livebirth rate per transfer during the funded period.

Interestingly, this occurred despite an increase in the cycle cancellation rate. Although it is possible that the dramatic increase in the number of cycles performed without time to effectively increase capacity may have initially stressed the ability of IVF centres to expand and continue to provide the same high-level care, the types of patients seeking treatment also changed. Funding altered the balance between the cost of trying another cycle and the probability that the next cycle might succeed. Increasingly, patients with a poor prognosis returned for another cycle rather than considering alternatives, such as egg donation or adoption. Moreover, because government funding applied separately to ovarian stimulation, egg collection and embryo transfer, both patients and IVF centres had no real disincentive to start IVF cycles even if they were cancelled prior to retrieval, or cancelled after retrieval. Thus, it was not unusual for patients with a poor prognosis to initiate multiple stimulated IVF attempts before finally completing all of their funded embryo transfers.
The impact on the success rate per transfer in FET cycles is interesting. The data show that the proportion of SET increased even more for FET cycles than fresh IVF cycles. From 2009 to 2016, the use of blastocyst transfer increased. Coupled with the fact that cryopreservation was included in the overall cycle reimbursement, the years 2010–2015 saw more selective use of embryo cryopreservation and higher subsequent cryo–thaw transfer success rates. These factors may have mitigated the negative impact of funding on the success rates of frozen–thaw cycles compared with fresh cycles.

In terms of the use of donor gametes within IVF cycles, a decrease in donor egg use during the programme was noted for all patient age groups. In terms of the use of donor eggs in Canada, it is important to note that, under Federal law, it is illegal to reimburse donors for their donation, and therefore almost all gametes from egg and sperm banks come from the USA. Although in the funded years, the purchase of donor sperm was covered by the programme, the purchase of donor eggs was not. Thus, those patients who may have most benefited from donor eggs had little to lose by attempting an IVF cycle using their own eggs, even when their prognosis was very poor. In a private setting, those patients may have chosen to put their money towards egg donation rather than spend it on an autologous attempt with a poor prognosis. As the data demonstrate, relatively few patients chose to spend additional money on treatments that were not covered in addition to or in place of the funded treatments. In terms of treatment age, although the law did not impose a maximum age limit, the clinics, using historical data from CARTR-BORN, applied a soft cut-off of 43 years of age for autologous IVF, so an increase in the use of donor eggs is seen after this age (49.2% of IVF cycles).
We have previously reported on the reduction in cost per live birth during the programme compared with previous years where a 50% tax deduction was possible in Quebec (Velez et al., 2014). It is important to note that the cost of the programme was the major reason cited for its untimely end. Although it is difficult to obtain precise details on the annual costs, they were widely presented in the media as being between $CAN 70 and 80 million per year. If one considers that the average lifetime contribution of an individual in terms of income tax in the province of Quebec is approximately $CAN 330,000 (StatsCanada), it can be estimated that the 9232+ babies born as a result of the programme will contribute over $CAN 3 billion. Therefore, at a cost of $CAN 400 million over the 5.5 years, it suggests that, in purely financial terms, the Provincial Government of Quebec achieved close to an eight-fold return on their investment.

The design of the coverage encouraged the cancellation of cycles as only a cycle resulting in an embryo transfer counted towards the three insured cycles per patient. This is clearly demonstrated by the significant increase in cycle cancellations in all age groups. This clearly had an important impact on results as well as financially on the programme. Such a design leads to aggressive rejection of suboptimal embryos since the concept that a better outcome can be achieved in a future cycle leads to the desire to maximize the use of the three available embryo transfers. Furthermore, it creates pregnancy and livebirth rate per cycle data that are not comparable with other studies. Undoubtedly counting a cycle from the start of ovarian stimulation or egg collection would reduce cancellation rates. Data from 2016, when the cancellation rate returned to <20%, confirms that this increased cancellation rate was associated directly with the funding design, and not, as could have been hypothesized, by development of new technologies such as time lapse or
pre-implantation genetic testing for aneuploidy which tend to ‘deselect’ embryos and can result in increased cancellation.

There is substantial variation from country to country regarding IVF funding. A survey of patients, professionals and the general public in Germany concluded that the majority of people supported public coverage of IVF. However, the concept of patient co-payments varied depending on which group was interviewed: 33% of patients agreed with this idea compared with 75% of professionals and the general public. At the time of publication, patients contributed 50% of the costs in Germany (Rauprich et al., 2010).

Some opponents to IVF funding have argued that infertility is a social rather than a medical issue (Hughes et al., 2009). However, infertility is defined as a disease by the World Health Organization (Zegers-Hochschild et al., 2009). Furthermore, due to an increased presence in the media as well as its inclusion as storylines in movies and television series, the use of IVF has become more widely known and understood by the general public, and the ability to access this technology is increasingly desired. Of course, the associated expense creates challenges for public funding.

There are certainly lessons that can be learned from Quebec’s experience with regard to the best manner in which a programme can be designed to maximize its potential whilst maintaining fiscal restraints. The study data suggest that funding design can influence the clinical results, as well as encourage changes in clinical practice. Based on this analysis, it is suggested that a programme should fund a limited number of egg collections per patient, should include all resulting fresh and frozen embryos, and should have strict inclusion/exclusion criteria.

Acknowledgements
The authors wish to thank the team at CARTR-BORN, especially Holly Ockenden for providing the data from the registry used in this analysis. The authors would also like to recognize the work of all the ART clinics in Quebec for their participation in CARTR, and their hard work, particularly during the Quebec ART programme.

References
Gazette Officielle du Québec, 2015. An act to enact the Act to promote access to family medicine and specialised medicine services and to amend various legislative provisions relating to assisted procreation. Chapter 25, 10 November 2015.

Declaration: The authors report no financial or commercial conflicts of interest.

Dr Francois Bissonnette is the Medical Director of OVO, a fertility clinic in Montreal, and a Professor in the Department of Obstetrics-Gynecology at the University of Montreal. He completed his specialty training in obstetrics and gynaecology in 1980, and was certified by the Royal College of Physicians and Surgeons of Canada in 1985. He was subsequently trained in reproductive endocrinology and infertility at the University of Louisville, Kentucky. He is a past President of the Canadian Fertility and Andrology Society, and is currently Director of the Canadian ART Registry.
Table 1. Cancellation rates of fresh in-vitro fertilization cycles between the insured period (2010–2015) and pre/post insured years (2009 and 2016).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>Total cycles started</td>
<td>2055</td>
<td>5353</td>
<td>7408</td>
<td>39,722</td>
</tr>
<tr>
<td></td>
<td>Cycles completed</td>
<td>1769</td>
<td>4380</td>
<td>6149</td>
<td>26,064</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>13.9%</td>
<td>18.2%</td>
<td>17.0%</td>
<td>34.4%</td>
</tr>
</tbody>
</table>
Table 2. Livebirth rates per embryo transfer (ET) for in-vitro fertilization (IVF), modified natural cycle IVF and frozen embryo transfers (FET) between the insured period (2010–2015) and pre/post insured years (2009 and 2016) in the province of Quebec.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n live births</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>1725</td>
<td>1398</td>
<td>3123</td>
<td>23,765</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2016</td>
<td>616</td>
<td>395</td>
<td>1011</td>
<td>6066</td>
<td></td>
</tr>
<tr>
<td>2009 and 2016</td>
<td>35.7%</td>
<td>28.3%</td>
<td>32.3%</td>
<td>25.5%</td>
<td></td>
</tr>
<tr>
<td>Natural IVF</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n live births</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>44</td>
<td>37</td>
<td>81</td>
<td>2062</td>
<td>0.03</td>
</tr>
<tr>
<td>2016</td>
<td>16</td>
<td>13</td>
<td>29</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td>2009 and 2016</td>
<td>36.4%</td>
<td>35.2%</td>
<td>35.8%</td>
<td>25.2%</td>
<td></td>
</tr>
<tr>
<td>FET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n live births</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>492</td>
<td>2938</td>
<td>3430</td>
<td>12,924</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2016</td>
<td>109</td>
<td>765</td>
<td>874</td>
<td>2824</td>
<td></td>
</tr>
<tr>
<td>2009 and 2016</td>
<td>22.2%</td>
<td>26.1%</td>
<td>25.5%</td>
<td>21.9%</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n live births</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>2261</td>
<td>3373</td>
<td>5634</td>
<td>38,955</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2016</td>
<td>741</td>
<td>1163</td>
<td>1904</td>
<td>9232</td>
<td></td>
</tr>
<tr>
<td>2009 and 2016</td>
<td>32.8%</td>
<td>34.5%</td>
<td>33.9%</td>
<td>23.7%</td>
<td></td>
</tr>
</tbody>
</table>

*<38 years old.
Table 3. Livebirth rates per embryo transfer (ET) using in-vitro fertilization with ovarian stimulation between the insured period (2010–2015) and pre/post insured years (2009 and 2016) in the province of Quebec.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 years</td>
<td></td>
<td></td>
<td>197</td>
<td>144</td>
<td>341</td>
<td>2708</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Total ET</td>
<td>n live births</td>
<td>93</td>
<td>60</td>
<td>153</td>
<td>968</td>
<td></td>
</tr>
<tr>
<td></td>
<td>%</td>
<td></td>
<td>47.2%</td>
<td>41.7%</td>
<td>44.9%</td>
<td>35.7%</td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>586</td>
<td>389</td>
<td>975</td>
<td>6754</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>246</td>
<td>140</td>
<td>386</td>
<td>2260</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42.0%</td>
<td>36.0%</td>
<td>39.5%</td>
<td>33.5%</td>
<td></td>
</tr>
<tr>
<td>35–38 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>483</td>
<td>402</td>
<td>885</td>
<td>6732</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>179</td>
<td>117</td>
<td>296</td>
<td>1760</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37.1%</td>
<td>29.1%</td>
<td>33.4%</td>
<td>26.1%</td>
<td></td>
</tr>
<tr>
<td>39–40 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>236</td>
<td>210</td>
<td>446</td>
<td>3288</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53</td>
<td>42</td>
<td>95</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22.5%</td>
<td>20.0%</td>
<td>21.3%</td>
<td>18.2%</td>
<td></td>
</tr>
<tr>
<td>41–43 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>181</td>
<td>209</td>
<td>390</td>
<td>3839</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32</td>
<td>25</td>
<td>57</td>
<td>409</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.7%</td>
<td>12.0%</td>
<td>14.6%</td>
<td>10.7%</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>Total ET</td>
<td>n live births</td>
<td>1725</td>
<td>1398</td>
<td>3123</td>
<td>23,765</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>616</td>
<td>395</td>
<td>1011</td>
<td>6066</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35.7%</td>
<td>28.3%</td>
<td>32.4%</td>
<td>25.5%</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Livebirth rates using in-vitro fertilization in a modified natural cycle between the insured period (2010–2015) and pre/post insured years (2009 and 2016) in the province of Quebec.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET n live births</td>
<td>10</td>
<td>1</td>
<td>11</td>
<td>316</td>
<td>0.76</td>
</tr>
<tr>
<td>%</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30.0%</td>
<td>100%</td>
<td>36.4%</td>
<td>32.0%</td>
<td></td>
</tr>
<tr>
<td>30–34 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET n live births</td>
<td>21</td>
<td>14</td>
<td>35</td>
<td>965</td>
<td>0.03</td>
</tr>
<tr>
<td>%</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>253</td>
<td></td>
</tr>
<tr>
<td></td>
<td>47.6%</td>
<td>35.7%</td>
<td>42.9%</td>
<td>26.2%</td>
<td></td>
</tr>
<tr>
<td>35–38 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET n live births</td>
<td>13</td>
<td>22</td>
<td>35</td>
<td>781</td>
<td>0.3</td>
</tr>
<tr>
<td>%</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>23.1%</td>
<td>31.8%</td>
<td>28.6%</td>
<td>22.2%</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total ET n live births</td>
<td>44</td>
<td>37</td>
<td>81</td>
<td>2062</td>
<td>0.03</td>
</tr>
<tr>
<td>%</td>
<td>16</td>
<td>13</td>
<td>29</td>
<td>520</td>
<td></td>
</tr>
<tr>
<td></td>
<td>36.4%</td>
<td>35.2%</td>
<td>35.8%</td>
<td>25.2%</td>
<td></td>
</tr>
</tbody>
</table>

ET, embryo transfers.
Table 5. Livebirth rates using frozen embryo transfers between the insured period (2010–2015) and pre/post insured years (2009 and 2016) in the province of Quebec.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30 years</td>
<td>Total ET</td>
<td>n live births</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>77</td>
<td>15</td>
<td>19.5%</td>
<td>352</td>
<td>121</td>
</tr>
<tr>
<td>30–34 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>194</td>
<td>47</td>
<td>24.2%</td>
<td>899</td>
<td>309</td>
</tr>
<tr>
<td>35–38 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>119</td>
<td>28</td>
<td>23.5%</td>
<td>904</td>
<td>269</td>
</tr>
<tr>
<td>39–40 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>51</td>
<td>11</td>
<td>21.6%</td>
<td>301</td>
<td>84</td>
</tr>
<tr>
<td>41–43 years</td>
<td>Total ET</td>
<td>n live births</td>
<td>34</td>
<td>3</td>
<td>8.8%</td>
<td>333</td>
<td>52</td>
</tr>
<tr>
<td>Overall</td>
<td>Total ET</td>
<td>n live births</td>
<td>492</td>
<td>109</td>
<td>22.2%</td>
<td>2938</td>
<td>874</td>
</tr>
</tbody>
</table>

ET, embryo transfers.
Table 6. Proportion of use of single embryo transfers (SET) between 2009 and 2010–2015 in the province of Quebec.

<table>
<thead>
<tr>
<th></th>
<th>2009</th>
<th>2010–2015</th>
<th>2016</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVF</td>
<td>Total ET</td>
<td>1769</td>
<td>26,064</td>
<td>1398</td>
</tr>
<tr>
<td></td>
<td>SET %</td>
<td>163</td>
<td>16,766</td>
<td>999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.2%</td>
<td>64.3%</td>
<td>71.5%</td>
</tr>
<tr>
<td>Natural IVF</td>
<td>Total ET</td>
<td>44</td>
<td>2299</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>SET %</td>
<td>42</td>
<td>2251</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95.0%</td>
<td>97.9%</td>
<td>100%</td>
</tr>
<tr>
<td>FET</td>
<td>Total ET</td>
<td>492</td>
<td>12,924</td>
<td>2938</td>
</tr>
<tr>
<td></td>
<td>SET %</td>
<td>49</td>
<td>9496</td>
<td>2546</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0%</td>
<td>73.5%</td>
<td>86.7%</td>
</tr>
</tbody>
</table>

IVF, in-vitro fertilization; FET, frozen embryo transfers; ET, embryo transfers.
Table 7. Multiple pregnancy rate from 2009 until 2016 in the province of Quebec.

<table>
<thead>
<tr>
<th>Year</th>
<th>Multiple pregnancy rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>25.6%</td>
</tr>
<tr>
<td>2010 (partial year)</td>
<td>3.7%</td>
</tr>
<tr>
<td>2011</td>
<td>7.0%</td>
</tr>
<tr>
<td>2012</td>
<td>6.0%</td>
</tr>
<tr>
<td>2013</td>
<td>3.3%</td>
</tr>
<tr>
<td>2014</td>
<td>3.4%</td>
</tr>
<tr>
<td>2015</td>
<td>5.2%</td>
</tr>
<tr>
<td>2016</td>
<td>4.5%</td>
</tr>
</tbody>
</table>
Figure 1
Figure 2
### FORM UPR16

**Research Ethics Review Checklist**

Please include this completed form as an appendix to your thesis (see the Research Degrees Operational Handbook for more information)

<table>
<thead>
<tr>
<th>Postgraduate Research Student (PGRS) Information</th>
<th>Student ID: UP884967</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PGRS Name:</strong></td>
<td>Simon Phillips</td>
</tr>
<tr>
<td><strong>Department:</strong></td>
<td>Pharmacy and Biomedical Sciences</td>
</tr>
<tr>
<td><strong>First Supervisor:</strong></td>
<td>Professor Graham Mills</td>
</tr>
<tr>
<td><strong>Start Date:</strong> (or progression date for Prof Doc students)</td>
<td>01-02-2018</td>
</tr>
<tr>
<td><strong>Study Mode and Route:</strong></td>
<td>Full-time ✓ MPhil MD Professional Doctorate</td>
</tr>
<tr>
<td><strong>Title of Thesis:</strong></td>
<td>Towards elective single embryo transfer: investigating approaches for improving accessibility and efficacy of in vitro fertilisation treatment and their implications for patients</td>
</tr>
<tr>
<td><strong>Thesis Word Count:</strong></td>
<td>8939 (excluding ancillary data)</td>
</tr>
</tbody>
</table>

If you are unsure about any of the following, please contact the local representative on your Faculty Ethics Committee for advice. Please note that it is your responsibility to follow the University’s Ethics Policy and any relevant University, academic or professional guidelines in the conduct of your study.

Although the Ethics Committee may have given your study a favourable opinion, the final responsibility for the ethical conduct of this work lies with the researcher(s).

### UKRIO Finished Research Checklist:

(If you would like to know more about the checklist, please see your Faculty or Departmental Ethics Committee rep or see the online version of the full checklist at: [http://www.ukrio.org/what-we-do/code-of-practice-for-research/](http://www.ukrio.org/what-we-do/code-of-practice-for-research/))

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Have all of your research and findings been reported accurately, honestly and within a reasonable time frame?</td>
<td>YES ✓ NO</td>
</tr>
<tr>
<td>b) Have all contributions to knowledge been acknowledged?</td>
<td>YES ✓ NO</td>
</tr>
<tr>
<td>c) Have you complied with all agreements relating to intellectual property, publication and authorship?</td>
<td>YES ✓ NO</td>
</tr>
<tr>
<td>d) Has your research data been retained in a secure and accessible form and will it remain so for the required duration?</td>
<td>YES ✓ NO</td>
</tr>
<tr>
<td>e) Does your research comply with all legal, ethical, and contractual requirements?</td>
<td>YES ✓ NO</td>
</tr>
</tbody>
</table>

### Candidate Statement:

I have considered the ethical dimensions of the above named research project, and have successfully obtained the necessary ethical approval(s)

**Ethical review number(s) from Faculty Ethics Committee (or from NRES/SCREG):**

N/A

If you have not submitted your work for ethical review, and/or you have answered ‘No’ to one or more of questions a) to e), please explain below why this is so:

PhD by Publication: Ethics approval obtained as required at time of each research project

UPR16 – April 2018