

**THE COST EFFECTIVE IVF
STRATEGIES IN ASSISTED
REPRODUCTION TECHNOLOGY
PROGRAMMES (ART)**

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**Dissertation presented for the degree of
Doctor of Philosophy at Stellenbosch University
Department of Obstetrics and Gynaecology
Faculty of Medicine and Health Sciences**

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December 2016

DECLARATION

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Date: December 2016

SUMMARY

CHAPTER I

Understanding the physiology of oocyte(s) recruited, selected and retrieved in a cycle of assisted reproductive technology (ART) is fundamentally important towards the development of the embryo with great potential for conception and live birth. This is discussed in detail.

More important is the interpretation and utilization of the scientific evidence in this chapter to explore less expensive methods of optimizing oocyte quality in mild ovarian stimulation in vitro fertilization (IVF).

CHAPTER II

Clomiphene citrate (CC) is an inexpensive and safe drug that can be used alone or in combination with gonadotropins in IVF.

Clinical outcomes in different IVF treatments using CC were reviewed and discussed in detail. The major concern regarding CC in ART is the risk of premature luteinizing hormone (LH) surge with subsequent detrimental effect on the oocyte quality. This issue is discussed with outlined strategies (inexpensive) to minimize the risk.

CHAPTER III

The effective methods to prevent premature LH surge in ART include gonadotropin releasing hormone antagonists (GnRHa) and gonadotropin releasing hormone agonists (GnRH). But these methods are expensive and unaffordable in resource-limited countries. We therefore performed a randomised controlled trial to evaluate a simple method of prolonged usage of CC as a strategy to prevent premature LH surge in ART treatment. The protocol is described in detail. The trial showed that prolonged usage of CC did not suppress premature LH surge in mild ovarian stimulation ART. But it motivated us to explore other inexpensive strategies for lowering the risk of premature LH surge such as pre-treatment with oral contraceptives, the use of tamoxifen and the use of progesterone during ovarian stimulation.

CHAPTER IV

In our endeavour to explore strategies to make ART accessible, a public-private interaction (PPI) model is described in detail, highlighting different areas where the cost of IVF can be significantly reduced. They include infrastructure and equipment, personnel, ovarian stimulation protocol (detailed in Chapters II and III) and modification in the laboratory routine regarding oocyte retrieval.

CHAPTER V

This meta-analysis compared mild ovarian stimulation IVF with conventional treatment in order to counsel patients appropriately. The study showed significantly better outcomes in terms of live birth rates and ongoing pregnancy rates per started cycle, all in favour of conventional stimulation IVF, which therefore currently remains the preferred treatment of choice.

CHAPTER VI

Understanding the physiology of folliculogenesis has made it possible to integrate mild ovarian stimulation in our unit ART programme at a low cost. (Chapter I)

Reassuring clinical outcomes of CC in ART also motivated the unit to maintain low cost of treatment with the use of safe and effective medication. (Chapter II)

The finding that prolonged usage of CC does not reduce the risk of premature LH surge has also allowed the unit to maintain the old protocol of 5 days' use, but motivated us to explore other inexpensive methods. (Chapter III)

The PPI model certainly managed to make ART treatment accessible to subfertile couples that would have never had a chance to be proud parents. (Chapter IV)

Because this model is feasible and can be implemented at a reasonably low cost, it presents a viable option to make ART accessible in resource-limited countries.

OPSOMMING

HOOFSTUK I

Dit is uiters belangrik om die fisiologie van oosiet(e) werwing, seleksie en onttrekking in 'n geassisteerde reprodktiewe tegnologie (ART) siklus te verstaan om 'n embrio met groot potensiaal vir konsepie en lewendige geboorte te ontwikkel. Dit word in meer detail bespreek.

Meer belangrik is die interpretasie en gebruik van wetenskaplike bewyse in hierdie hoofstuk om goedkoper metodes te ondersoek om oosiet kwaliteit met matige ovariële stimulasie in vitro bevrugting (IVB) te verhoog.

HOOFSTUK II

Klomifeen sitraat (CC) is 'n goedkoop en veilige middel wat alleen of in kombinasie met gonadotropiene in IVB gebruik kan word.

Kliniese uitkomst in verskillende IVB behandelings met CC is ondersoek en in detail bespreek. Die grootste bekommernis rakende CC in ART is die risiko van voortydige LH styging met daaropvolgende nadelige invloed op die oosiet kwaliteit. Dit word bespreek met 'n verduideliking van strategieë (goedkoop) om die risiko te verminder.

HOOFSTUK III

Effektiewe metodes om voortydige LH styging in ART te voorkom sluit gonadotropien vrystellende hormoon antagonist (GnRHa) en gonadotropien vrystellende hormoon agoniste (GnRH) in. Hierdie metodes is egter duur en onbekostigbaar in lande met beperkte hulpbronne. Ons het dus 'n gerandomiseerde gekontroleerde studie uitgevoer om 'n eenvoudige metode van verlengde gebruik van CC te ondersoek as 'n strategie om voortydige LH oplewing in ART behandeling te voorkom. Die protokol is in detail bespreek. Die studie het bevind dat langdurige gebruik van CC nie voortydige LH styging met matige ovariële stimulasie ART onderdruk het nie. Dit het ons egter motiveer om na ander goedkoop maniere te kyk om die risiko van voortydige LH oplewing te verminder, soos vooraf behandeling met orale voorbehoedmiddels, die gebruik van tamoksifeen en die gebruik van progesteron gedurende ovariële stimulasie.

HOOFSTUK IV

In ons poging om metodes te ondersoek om ART toeganklik te maak, word die publieke-privaat interaksie (PPI) model breedvoerig beskryf met die klem op verskillende areas waar die koste van IVF aansienlik verminder kan word. Dit sluit in infrastruktuur and toerusting, personeel, ovariële stimulasie protokol (verduidelik in Hoofstukke II en III) en aanpassing van laboratorium roetine betreffende die onttrekking van oosiete.

HOOFSTUK V

Hierdie meta-analiese het matige stimulasie IVF met gebruiklike behandeling vergelyk sodat pasiënte deeglik ingelig kon word. Die studie het merkbaar beter uitkomst in terme van lewendgebore syfers en voortgaande geboorte syfers per aanvang siklus, almal ten gunste van gebruiklike stimulasie, getoon wat tans die behandeling van keuse bly.

HOOFSTUK VI

Om die fisiologie van follikulogenese te verstaan het dit moontlik gemaak om matige ovariële stimulasie in ons eenheid se ART program te integreer teen 'n lae koste. (Hoofstuk I)

Gerusstellende kliniese uitkomst van CC in ART het ook die eenheid motiveer om 'n laekoste behandeling te handhaaf met die gebruik van veilige en effektiewe medikasie. (Hoofstuk II)

Die bevindinge dat langdurige gebruik van CC nie die risiko vir voortydige LH styging verminder nie het ons eenheid in staat gestel om 'n ou protokol van 5 dae gebruik te handhaaf, maar ons gemotiveer om ander goedkoop metodes te ondersoek. (Hoofstuk III)

Die PPI model het beslis ART behandeling toeganklik gemaak vir subfertiele egpare wat geen kans sou hê om trotse ouers te word nie. (Hoofstuk IV)

Omdat hierdie model haalbaar is en dit uitgevoer kan word teen 'n redelike lae koste, skep dit 'n lewensvatbare opsie om ART toeganklik te maak in lande met beperkte hulpbronne.

ACKNOWLEDGEMENTS

First of all, I would like to thank God Almighty for the strength and direction through this journey. It was through “The Prayer of Jabez” that we completed this journey, and by living and performing every day of our work according to the following scripture. *Peter 4:10: “As each has received a gift, use it to serve one another as good stewards of God’s varied grace.”*

I would like to thank my parents for their love and teachings all these years to mould the person I have become.

It is with a deep sense of appreciation to my wife, Tselane, for her love and support from the beginning up to now. I would truly like to say “Thank you”.

To my children, Onalenna and Orapeleng, thank you for understanding whenever I request time to work at the expense of your precious family time.

To my mentor, my inspiration and my role model, Prof TF Kruger, thank you very much for your leadership, guidance and encouragement. Above all, thank you for believing in me. I will for ever respect you for the human being you are and the ethos you live by.

Igno and Kobie, thank you for the generosity and the support from the very first day we met. It was comforting to know I could talk and seek advice from you.

Prof Theron, your spirit of human kindness (“Ubuntu”) and visionary leadership have been the force of encouragement, and I thank you.

The “Two Musketeers”, Evelyn and Marie-Lena, your hard work, commitment and passion has been a pillar of strength to me and the young scientists who join the family, thank you very much.

To the Vincent Pallotti team of scientists, sisters and administrators, from the bottom of my heart, thank you for your willingness to help at all times. To get the programme off the ground would not have been possible without your involvement from the beginning.

Erna and Madaleine, thank you very much for your impressive work ethic, it is encouraging to work with such a team.

The Tygerberg Gynaecology Clinic, operating theatre (S and T) and the GEK theatre team, your individual contribution is greatly appreciated.

To my past mentors and teachers, thank you for the valuable lessons you imparted.

Lastly, I would like to dedicate this work to Frik (Stander), the man with such an amazing work ethic and sense of pride in his work. Frik, without you this baby would have never been born and I thank you. And to the men and women dreaming of being proud parents one day, we trust this work will help you come close to living your dreams. (*Psalms 23:1-6*)

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LIST OF ABBREVIATIONS

AFC – antral follicle count

AMH – anti Mullërian hormone

AR – androgen receptors

ART – assisted reproductive technology

BMI – body mass index

BMP – bone morphogenic protein

CL – corpus luteum

COH – controlled ovarian hyperstimulation

CPR – clinical pregnancy rates

DHEA – dehydroepiandrosterone

ET – embryo transfer

GnRHa / GnRH – gonadotropin releasing hormone agonists / antagonist

GDF – growth differentiation factor

GIFT – gamete intrafallopian tube transfer

FSH – follicle stimulating hormone

GH – growth hormone

hCG – human chorionic gonadotropins

hMG – human menopausal gonadotropins

ICSI – intracytoplasmic sperm injection

IGF – insulin-like growth factor

PGFBP – insulin-like growth factor binding protein

IVF – in vitro fertilization

LBR – live birth rates

LH – luteinizing hormone

mRNA messenger RNA

NSAIDs – non-steroidal anti-inflammatory drugs

OCP – oral contraceptive pill

OHSS – ovarian hyperstimulation syndrome

OPR – ongoing pregnancy rates

PCOS – polycystic ovarian syndrome

PPI – public-private interaction

RCT – randomised controlled trial

r-hLH – recombinant luteinizing hormone

rFSH – recombinant Follicle stimulating hormone

TGF- β – transforming growth factor-beta

FMHS – Faculty of Medicine and Health Sciences

HAS – Human Serum Albumin

CHAPTER 1: FOLLICULOGENESIS

SYNOPSIS

The quantity and quality of oocyte(s) recruited, selected and retrieved in a cycle of ART is fundamentally important to the development of the embryo with great potential for conception and live birth.

Follicular development and folliculogenesis is a dynamic structural and endocrinological process that has to be well orchestrated by two-cell, two-gonadotropin theory including FSH and LH together with steroidal hormones and non-steroidal paracrine factors such as TGF- β superfamily and IGF systems. It is understood that the duration of the rise in FSH above a critical threshold determines the number of dominant follicles to be selected from the recruited cohort. However the exact mechanism on the way in which follicular reserve is controlled and how follicles enter this growth journey of recruitment and selection towards ovulation or atresia, is not well understood. Furthermore, the emerging evidence in support of multiple (two or three) antral follicular waves recruited in a menstrual cycle still needs to be evaluated for clinical relevance, especially in ART treatment cycles.

The role of adjunct therapy in ART cycles to improve pregnancy outcomes lacks robust evidence to be recommended as routine co-treatment during ovarian stimulation. The apparent significant benefit of other adjunct therapies has been shown in women with poor ovarian response, unfortunately leaving many questions unanswered when it comes to the effect of adjunct therapies in women with normal ovarian response.

Understanding how the ovary functions as a unit is therefore very important in controlled ovarian hyperstimulation cycles. More so, investigating ways and strategies on how to improve the quality of oocytes retrieved per cycle is also crucial to improving ART success rates.

Keywords: antral follicles, ovary, folliculogenesis, assisted reproductive technology, adjunct therapies

INTRODUCTION

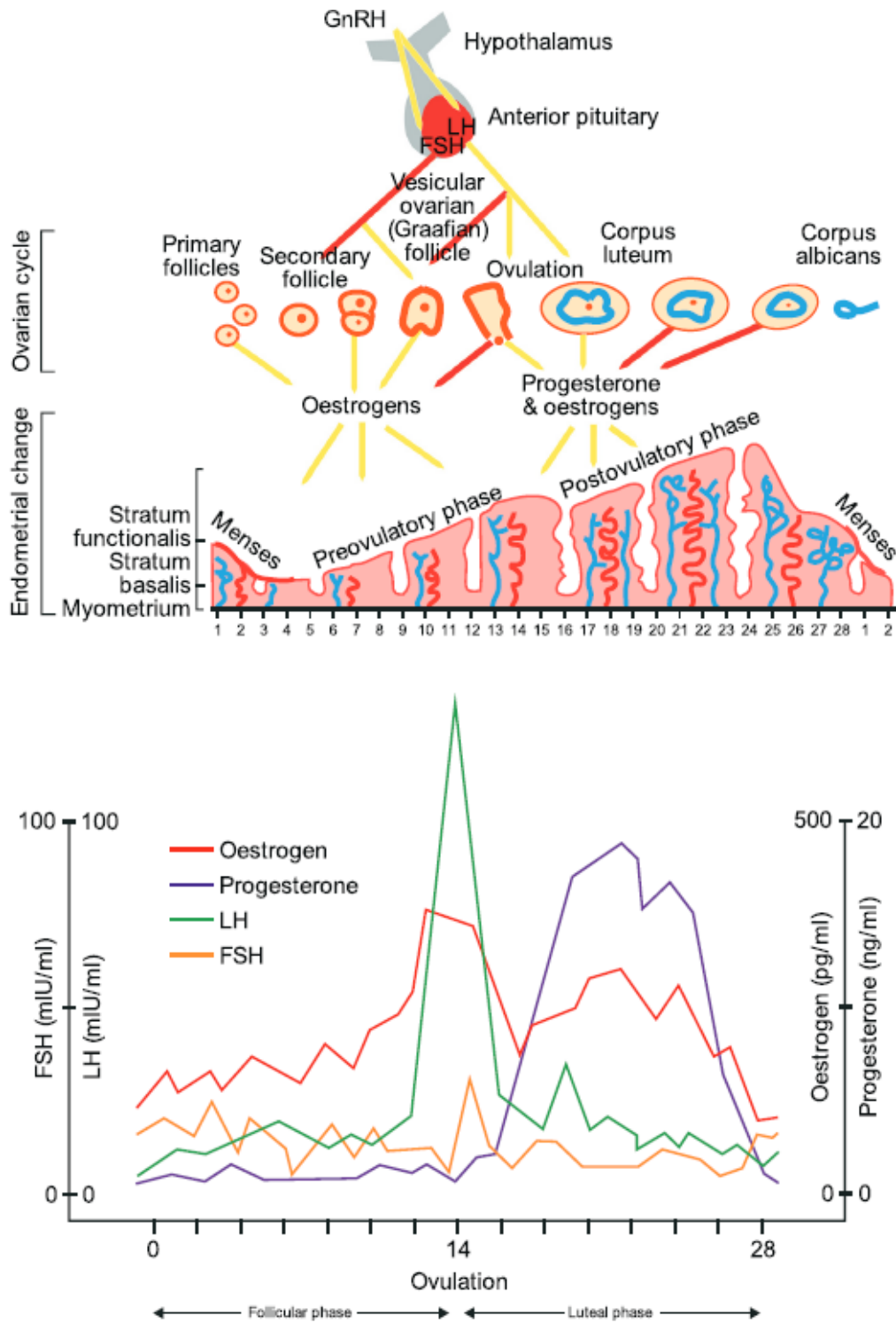
The art of folliculogenesis is crucial and fundamental in understanding the ovarian function for the purpose of menstrual disorder and infertility management. The ovary as the unit contains oocytes that may eventually lead to embryo formation and conception following the fertilization process. It also provides the steroid and protein hormones that are essential for ovarian and menstrual regulation [1]. William Harvey proclaimed “**ex ovo omnia**” – all things come from the egg – emphasising the importance of the ovary [2].

With all the knowledge acquired over the decades, the human ovarian follicular growth and regression remains a complicated physiological phenomenon for scientists and clinicians alike. The current knowledge is based on the synergistic use of histologic, endocrinologic and ultrasonographic modalities [3,4] including extrapolation from studies performed in non-human primates, farm animals and rodents [4]. More studies, particularly in human ovarian function, are therefore still required.

Physiology of follicular development and oocyte maturation

During the menstrual cycle the ovarian follicles undergo dynamic morphologic and endocrinologic changes.

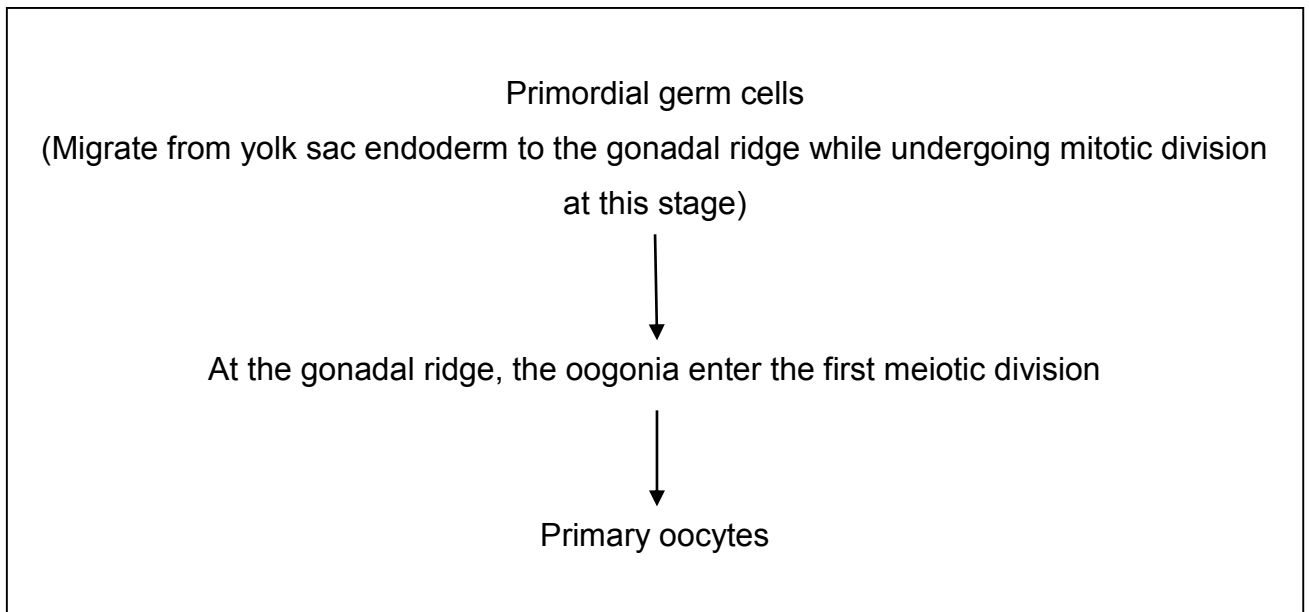
Figure 1: Ovarian changes during menstrual cycle



(Adapted from Ezcurra et al., 2014, *Reprod Bio Endocrinol*, 12: 95)

Histologic studies of ovaries show that the entire duration of human folliculogenesis from the primordial phase to the pre-ovulatory phase is estimated to be approximately >175 days [5] and follicular development begins as early as the fourth month of the foetal life. [4]

Figure 2



Somatic cells originating from the primitive gonad (superficial epithelial cells, follicular granulosa cells, theca cells, interstitial cells and fibroblasts) surround the oogonia, forming a rudimentary ovarian follicle.

Follicles containing oocytes arrested in the dictate stage of meiosis I constitute the ovarian follicular reserve and the number of follicles occupying this reserve is estimated to be approximately 7 million at 20 weeks of gestation and approximately 1-2 million at birth [6]. Depletion of the ovarian follicular reserve begins during foetal life and continues throughout woman's lifetime via process of follicular atresia [7]. The pre-antral follicles of 0.1 – 0.2 mm develop independent of gonadotropins support [4].

Folliculogenesis – clarity on terminology [8]

Recruitment used to describe:

- (i) Initial transition of primordial follicles from the resting pool into the pre-antral growth phase;
- (ii) Cyclic recruitment of a cohort of antral follicles of 2-5mm during the menstrual cycle;
- (iii) The preferential growth of the dominant ovulatory follicle. The current understanding and acceptable description of recruitment is the emergence of a group or cohort of medium-sized (2-5mm) antral follicles [4].

Selection used to describe:

- (i) The recruitment of a cohort of 2-5mm antral follicles
- (ii) Preferential growth of a species – specific number of large antral follicles from the recruited cohort – dominant follicle selection.
The current acceptable description is the preferential growth of the dominant follicle from the cohort of recruited antral follicles [4].

Subordinate follicles, also known as challengers, ordinary or subdominants, compromise all follicles of the recruited cohort excluding the dominant follicle.

Three theories of follicular recruitment [4]

Continuous recruitment I

Animal studies concluded that early antral follicle growth occurred continuously throughout the estrous / menstrual cycle, while human studies suggest that small antral follicles of $\leq 4-6\text{mm}$ are recruited to grow continuously at all stages of reproductive life independent of gonadotropin support. Cyclic increases in the number of antral follicles have been observed at regular intervals during the menstrual cycle of women [9].

Single recruitment episode II

Histologic, endocrinologic and early ultrasonographic studies have demonstrated that a cohort of 2-5mm follicles is recruited from a continuous supply of antral follicles once during the menstrual cycle [10].

Endocrinologically, following the regression of CL, oestradiol and inhibin falls as a result the circulating FSH increases. The rise in FSH is thought to be responsible for preventing atresia of a cohort of 2-5mm antral follicles. [10] Similarly to the notion of preventing atresia, is the concept that recruitment is induced by rising FSH [11].

Inhibin B produced by granulosa cells in follicles of the recruited cohort acts in the endocrine manner to inhibit continued FSH secretion in the mid-follicular phase [12,13].

Inhibin A levels are low during the follicular phase but higher during the mid-luteal phase, suggesting that the CL is a source of inhibin A [13].

High oestradiol and inhibin A concentrations in the mid-luteal phase are thought to suppress FSH and thereby prevent the development of healthy follicles [14].

However, Pache *et al.* disputed the concept of a single increase in the number of 2-5mm antral follicles during the late luteal or early follicular phases by showing that the mean number of antral follicles, AFC (2-5mm) was not different in the early follicular, late follicular and luteal phases [10,15].

AMH is produced by the granulosa cells of the primary, secondary, pre-antral and early antral follicles (≤ 4 mm). AMH inhibits the initiation of primordial follicle growth from the ovarian reserve, thereby regulating the recruitment of antral follicles.

Multiple Follicular waves III

Multiple cohorts or “waves” of antral follicle recruitment have been described, suggesting that antral follicles may not only be seen in the late luteal phase [3,9,16].

Ultrasonographic studies have also demonstrated two waves of follicle development in women with regular menstrual cycles [9]. It is interesting to note that 68% of women exhibited two waves of follicle recruitment during the interovulation interval (IOI) while the remaining 32% exhibited three waves [4].

Important research questions to answer are:

- a. When is the appropriate time to assess the AFC to predict response to ovarian stimulation – could it be any time of the menstrual cycle??
- b. Can ovarian stimulation therapy be initiated at any time during the cycles? Including the luteal phase of the menstrual cycle?
- c. Innovative contraceptive designs?
- d. Do women with multiple follicular waves reach the menopause earlier or not?

Another observation is the inverse association between the circulating FSH and the number of follicles in a wave, and this is consistent with age-related decrease in AFC and elevated FSH in women [17].

Theories on Follicle Selection

Follicle selection is a process by which a single “dominant” follicle is chosen from the recruited cohort or wave for preferential growth [4,10,16]. It generally occurs in the early to mid-follicular phase of the menstrual cycle.

Follicle divergence

At the time of selection, the dominant follicle begins to “diverge” as it continues to grow while the subordinate follicles undergo atresia [18]. Divergence occurs when the dominant follicle reaches a diameter of approximately 10mm on day 6-9 of the follicular phase in women [10,16,18]. It is postulated that the future dominant follicle may contain more granulosa cells and FSH receptors, making it more sensitive to even low levels of circulating FSH compared to subdominant follicles, intra-ovarian manipulation [11].

Follicle dominance

Once the dominant follicle is formed, it exerts morphological and functional dominance.

High levels of FSH are required for the recruitment of a follicular cohort. The recruited cohort produces oestradiol and inhibin B from granulosa cells. They both suppress FSH, resulting “post surge” decline which is a crucial step in the selection process.

The duration of the rise in FSH above the critical threshold determines the number of dominant follicles selected from the recruited cohort [19]. This phenomenon is termed the “FSH Threshold” or “FSH Window” or “FSH gate” [19]. During ovarian stimulation therapy, prolonging the FSH window allows multiple follicles to be selected [19].

On day 5-8 of the menstrual cycle the aromatase activity begin in the granulosa cells of follicles larger than 6-8mm, with the dominant follicle producing more oestradiol than other follicles in the cohort [20]. Elevated oestradiol further suppresses FSH to the detriment of subordinate follicles but favours the dominant follicle with a large number of FSH receptors. Furthermore, the oestradiol secretion will result in LH receptor formation on the granulosa cells of the dominant follicle, therefore becoming less dependent on FSH and more responsive to LH [21].

Paracrine factors in folliculogenesis

Transforming growth factors– β superfamily

The TGF– β superfamily includes inhibin, activin, follistatin, TGF- β , BMP, GDF and AMH. Oocyte and cumulus cells have a special communication through the paracrine and/or autocrine mechanism to regulate antral follicle development and oocyte competence [4].

The role of inhibin B produced by granulosa cells and subsequently decreasing FSH production prior to dominant follicle selection, is known from animal studies [22]. Activin has been reported to be associated with an inhibitory effect on the LH-induced production of progesterone, preventing spontaneous luteinisation in mature antral follicles [23,24].

In contrast, follistatin and inhibin A are associated with increased LH-induced thecal androgen production, which serves as a substrate for dominant follicle oestradiol [25]. Therefore a programmed and systematic transition from an inhibin B/activin follicular environment to a follistatin/inhibin A environment is critical for dominant follicle development in women [26].

Insulin-like growth factor system

IGF I and II mRNA have been detected in the theca cells of the small antral follicles, but only IGF II mRNA has been detected in the granulosa cells of the dominant follicle [27], especially at the time of selection. IGF II and IGF I stimulate aromatase activity, oestradiol and progesterone production in human granulosa cells and promote androgen production in the theca cells of the growing dominant follicle [4]. In subordinate follicles IGF is sequestered by IGFBP-4, thereby inhibiting steroidogenesis in granulosa and theca cells, leading to atresia [28].

Pre-ovulatory follicle

The dominant follicle may grow to a follicle of 16-29mm [9]. The ovulatory follicle grows at a rate of 1-4mm/day [10]. During the mid-follicular phase, the dominant follicle is associated with increased aromatase activity and a rapid rise in circulating and follicular oestradiol-17 β [16]. Oestradiol production from the dominant follicle peaks the day before the LH surge (Fig. 1), providing positive feedback at the hypothalamus and pituitary to stimulate the surge of LH necessary for inducing ovulation [4]. Ovulation will therefore occur on average within 24hrs of the LH peak [29].

Ovarian stimulation in assisted reproductive technology and folliculogenesis

In COH cycles, the hypothalamus–pituitary–ovarian unit and the antral follicles are manipulated at the time of the selection process in the divergence phase [18,30,31].

The key question to be answered is: If the concept of multiple follicular wave emergences is understood and accepted, will it influence the strategies for synchronizing follicles in COH or the way the treatment is initiated [3]? Other authors

have proposed such change in the approach to treatment, especially in reproductive ageing woman, citing no risk of premature LH surge, and reduced cost of ART [3].

Oocyte quality and adjuncts in folliculogenesis

Oestrogen administration

Oestrogen administration for follicular synchronization has been evaluated with no overall benefit in improving ART outcomes [32,33]. The treatment would be initiated in the luteal phase, from day 20 of the previous cycle to day 2 of the following cycle at a dose of 4mg per day. The aim would be to lower the FSH levels during the luteo-follicular transition in order to increase the number oocytes for retrieval, reduce cancellation rates and increase fertilization rates, making it the less expensive option versus the long GnRH agonist down regulation protocol [31].

Oral contraceptive pill

It has been suggested that OCP pre-treatment in IVF cycles might be beneficial in improving ovarian response through inhibition of intrinsic gonadotropins before ovarian stimulation [34]. However, this was associated with poorer pregnancy outcomes even though there was a markedly reduced risk of ovarian cysts [35]. In a recent review the use of OCP pre-treatment in ART has been categorised as a promising intervention [36].

Androgen supplementation

Androgens (DHEA, testosterone and androstenedione) exert their action mainly through ARs [37]. ARs are expressed in all cell types of the ovarian follicle including the oocyte, granulosa cells and theca cells [38].

The low ovarian reserve has been associated with impaired quantity and quality of the ovarian follicles [37]. A number of studies suggest that androgen supplementation may enhance fertility potential in women with a low ovarian reserve [39,40,41].

The recommended dosage of DHEA of 75mg/day in women with a low ovarian reserve was associated with a high oocyte yield and significantly increased birth rates [39]. Furthermore it is suggested to be associated with lowering the risk of age-related aneuploidy [42] and miscarriages [43]. The exact mechanism is unclear but it is

suggested DHEA is a precursor for sex steroid hormones in the ovarian follicle and it may also induce FSH receptors in the granulosa cells [44]. DHEA also increases IGF I and decreases IGFBP-I, positively favouring follicular development [44]. Some authors do not believe the mechanism is responsible for the improvement in IVF treatment outcomes through the recruitment of more pre-antral follicles or very small antral follicles, as there was no change in AMH and inhibin B levels, but rather the rescue from atresia of small antral follicles as evidenced by an increase in AFC [45]. In a review and meta-analysis that included three trials of 153 women in a transdermal patch of testosterone group and 112 in the control group, they found significantly higher live birth rates (RR 1.91, 95%CI 1.01 to 3.63), with no difference observed in the number and quality of oocytes retrieved [46].

The limitation of evidence in androgen supplementation lay in the small sample sizes, lack of proper randomization and extrapolation from animal studies which might not be reproducible in humans [47,48]. This suggests the need for more trials on the role of androgens in ART outcomes and particularly in normal responders.

Stimulation protocols

Controlled ovarian hyperstimulation is an integral part of assisted reproduction with strong emphasis on the development of multiple follicles for retrieval of good quality oocytes for fertilization, implantation and overall live birth.

The stimulation protocols have evolved over the past decades with the introduction of pituitary down regulation regimes (GnRH agonists and antagonists) to prevent premature luteinisation and reduce high cycle cancellation rates [49]. The current evidence has so far concluded that no one protocol is superior to another [49,50]. The long GnRH analogues however appear to be more effective in poor responder women undergoing IVF and the antagonists are associated with significant reduction in OHSS [36,51].

With regard to different drug regimes, there is no difference in effect on overall LBR and clinical pregnancy rates [36,52,53]. Some authors have reported a potential concern with urinary hMG, namely that it possesses hCG that would lead to excessive LH-like activity resulting in premature luteinisation and reduced fertilization rates [54].

However this concern has not been confirmed in robust literature and it will have to be evaluated in larger clinical trials.

Luteinizing hormone supplementation

According to the two-cell two-gonadotropin theory, the FSH and LH together with local steroidal and non-steroidal factors stimulate follicular growth and maturation, ovulation and the development of the CL [1]. Several studies have reported better oocyte and embryo quality, reduction in the apoptosis rate, and improved fertilization, implantation and pregnancy rates with overall improvement in the ART outcomes in women treated with r-hLH in IVF/ICSI treatment [55-58].

However, some authors did not find any benefit in terms of increased pregnancy rates [59,60]. The apparent overwhelming effect of LH supplementation is reported in poor responders with a reduced rate of early pregnancy loss, increased number of oocytes retrieved and significantly higher clinical pregnancy rates [60-62].

In a recent Cochrane review, the use of r-hLH in ART cycles is categorized as a promising intervention, with more evidence still required [36].

Glucocorticoids

Glucocorticoids are thought to stimulate GH and IGF 1 during ovarian stimulation [63]. Keay *et al* found that low-dose dexamethasone co-treatment, 1mg/day from the day of initiation of gonadotropins until the night before oocyte retrieval, was associated with reduction in poor ovarian response, but the mechanism by which glucocorticoids alter the ovarian responsiveness remains unclear [63]. It has further been postulated that glucocorticoids may be used as an immunomodulator by lowering the natural killer cells to normalise the cytokine expression profile in the endometrium in order to improve implantation [64]. In a Cochrane review by Boomsma and colleagues, there was no clear evidence that the administration of peri-implantation glucocorticoids improved implantation rates and ART outcomes [64]. However, they further reported borderline statistically significant improvement in pregnancy rates in women undergoing IVF and not ICSI. The reason for this finding was unclear [64].

Limited available evidence has shown improvement in ovulation rates, fertilization and pregnancy rates when glucocorticoids are used in combination with CC, versus CC alone [49]. Given the lack of good evidence, the role of dexamethasone as an adjunct in ART still needs to be investigated before it can be recommended as a routine treatment in ART treatments.

Metformin

There is no conclusive evidence that metformin before or during treatment of ART cycles improved LBR in women with PCOS. However it is associated with increased clinical pregnancy rates and reduced risk of OHSS [36,65,66].

Aspirin

Low-dose aspirin is supposed to increase the ovarian and uterine blood flow with subsequent increase in ART outcomes, but this benefit has not been proven in terms of live birth and clinical pregnancy rates when compared with placebo or no treatment [36,67].

Growth hormone

The use of a growth hormone as an adjunct therapy has been reported to be associated with significantly increased LBR in poor responders in ART treatment, therefore recognizing the intervention as effective in that regard [36].

SUMMARY AND CONCLUSION

With no reasonable doubt, many milestones have been achieved in ART [68], and the science of ovarian stimulation is certainly one of them. However, as we strive for better ART outcomes and yet being mindful of the possible complications such as OHSS and the increased cost of treatment, refined and robust knowledge on folliculogenesis is still necessary.

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CHAPTER 2: CLOMIPHENE CITRATE IN ASSISTED REPRODUCTIVE TECHNOLOGIES: WHAT IS THE FUTURE? A NARRATIVE REVIEW

SYNOPSIS

Objective: To evaluate the role of clomiphene citrate (CC) alone or in combination with gonadotropins with/without antagonists in ART programmes.

Design: Narrative review

Search methods: We reviewed the literature involving the use of CC alone (publications after 1990) and CC in combination with gonadotropins with or without mid-cycle GnRH antagonists (publications after 2000) in IVF/ICSI cycles. The search was electronically using PubMed Central, Medline, and Embase and the reference lists of articles. Relevant conference proceedings and other articles were hand searched.

Selection criteria and outcome measures: All studies that involved CC alone or in combination with gonadotropins with/without mid-cycle antagonists in IVF/ICSI were included. The following outcomes were measured: number of oocytes retrieved, number of embryos transferred, cryopreserved cycles, endometrial thickness, rates of premature LH surge ($LH \geq 10IU$), clinical pregnancy rates, OPR and LBR.

Main Results: A total of thirty studies were included in the review. There were nine studies in CC alone, of which three were RCTs. Twenty one studies involved CC in combination with gonadotropins, six no mid-cycle antagonist and 15 mid-cycle antagonist. Reported CPR in CC alone varied from 16% per cycle to 34% per ET. In CC plus gonadotropins cycles (without antagonists), CPR also varied from 14% per cycle to 42% per ET. The premature LH surge was as high as 30%. Cycles involving CC plus gonadotropins (with antagonists) reported LBR of 30-36% with no cases of premature LH surge observed.

Conclusion: The current available evidence does suggest a CC + gonadotropin + mid-cycle antagonist protocol as an effective protocol with comparable LBR and OPR.

In addition, it is associated with a reduced risk of OHSS and lesser number of gonadotropins required. CC + gonadotropins without antagonists also appears to be a feasible protocol in a well selected group of patients, young and normal endocrine profile. The risk of premature LH surge may be minimised by pre-treatment OCP or prolonged use of CC during stimulation or simultaneous use of CC and gonadotropins.

Keywords: Clomiphene citrate, premature LH surge, ART, mid-cycle GnRH antagonists, pregnancy outcomes

INTRODUCTION

The primary objective of COH in ART is to achieve multi-follicular development in order to retrieve mature oocytes that are competent for fertilization and may possibly result in pregnancy and eventually a live birth.

The long complex GnRH agonist and gonadotropin stimulation protocol is regarded as the gold standard regimen because it is highly effective in pituitary down regulation, thereby enabling synchronised development and the retrieval of a large number of oocytes per cycle with high pregnancy rates, fewer cancellation rates and better planning of treatment cycles [1].

However, this protocol is associated with increased cost of medication, increased risk of OHSS and a high order of multiple pregnancies [2,3].

Mild ovarian stimulation for IVF is defined as a procedure in which the ovaries are stimulated with gonadotropins and/or other oral compounds such as CC, with the intent to limit the number of oocytes obtained to fewer than seven [4]. They are currently being explored and receiving a lot of attention as an alternative strategy to minimise the risk of adverse events associated with complex conventional ovarian stimulation protocols.

Available data show that mild ovarian stimulation lessens the patient's discomfort [5] and is also associated with significantly lower doses of gonadotropins used per cycle [6,7]. But it is also associated with a low number of oocytes retrieved and embryos generated for transfer [7]. Of interest is that even though the number of embryos is lower in mild stimulation cycle, the proportion of chromosomally normal embryos is significantly increased [7]. The general disadvantages of mild ovarian stimulation include lower pregnancy rates and high cancellation rates per stated cycle [8-10].

CC was the first agent to be used in ovarian stimulation for IVF in the early 80's and it continues to be part and parcel of modern ART [11], particularly in mild regimens. Over and above known disadvantages associated with mild ovarian stimulation protocols, CC treatment is attributable to a 15-25% risk of premature LH that may result in

premature ovulation and loss of oocytes, and it also negatively affects the quality of oocytes and as a result, lowers the pregnancy rate [11,12].

Other than effective but long and complex GnRH agonist protocols, several strategies are available to prevent premature LH surge, such as mid-cycle GnRH antagonist usage [12-14]. However, Tavaniotou *et al.* reported significantly high levels of LH concentrations despite the administration of a GnRH antagonist [15]. Similar findings were reported by Engel and colleagues [16].

Hot flushes, lower abdominal pains, headaches and some psychic psychiatric symptoms have also been associated with the use of CC [17]. Furthermore, non-specific birth defects were also observed amongst CC users [18], but a review by Gibreel *et al.* did not find any association with birth defects [19].

The anti-oestrogenic detrimental effect of CC on the cervical mucus and the endometrium are postulated mechanisms resulting in poor pregnancy rates despite successful ovulation induction [20-22]. It is noted that in ART cycles involving the use of a CC/gonadotropins/mid-cycle antagonist versus a conventional long GnRH agonist protocol, there was no significant difference in the endometrial thickness (mm) between the two groups [23,24].

CC alone or in combination with gonadotropins have been suggested and provided as an alternative cost effective strategy for women with compromised ovarian reserve and poor response to conventional ovarian stimulation [24-26]. Therefore, because of the potential benefit in a selected group of patients, the well-known side effect profile, ease of administration and low cost results in CC remaining a useful agent in ovarian stimulation for IVF/ICSI treatment.

We therefore reviewed the literature involving the use of CC alone (publications after 1990) and CC in combination with gonadotropins with or without mid-cycle GnRH antagonists (publications after 2000) in IVF/ICSI cycles.

Design: Narrative review

Search methods:

We reviewed the literature involving the use of CC alone (publications after 1990) and CC in combination with gonadotropins with or without mid-cycle GnRH antagonists (publications after 2000) in IVF/ICSI cycles. The search was electronically using PubMed Central, Medline, and Embase and reference list of articles. Relevant conference proceedings and other articles were hand searched.

We reviewed articles that reported the following outcome measures:

- number of oocytes retrieved
- number of embryos transferred
- cryopreserved cycles
- endometrial thickness
- rates of premature LH surge ($LH \geq 10IU$)
- CPR (cardiac activity at 7 weeks)
- OPR (cardiac activity at 12 weeks)
- LBR (birth of singleton healthy baby)
- miscarriage rates (loss of pregnancy before 12 weeks of gestation)
- number of cycles cancelled, and
- total number of gonadotropins used.

Table 1, Part A: Clomiphene Citrate alone studies

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Saunders <i>et al.</i> 1992 [39]	Review	3377 patients undergoing IVF (1941) and GIFT (1436)	CC treatment cycle	Miscarriage rate significantly higher in CC group (24.4% IVF; 23.0 GIFT) vs. (20.7% IVF; 17.9% GIFT) in GnRHa group	Increased wastage due to CC or increased LH levels during folliculogenesis	Old study with missing detail.
MacDougall <i>et al.</i> 1994 [27]	Prospective Randomised	30 participants n=14 no treatment n=16 CC 100mg day2-6	CC vs NC	Increased no of oocytes in CC (1.8±0.3) CPR (18% CC vs. 0% NC) Significantly higher PR with CC Cycles cancelled in NC 10/14 (71%)	High cancellation rates in NC	Old data
Gentry <i>et al.</i> 1996 [30]	Prospective Comparative	128 patients but 84 evaluated for endometrial thickness (ETs)	CC	CPR in different ETs 3/15 (20%): >4mm <7mm 13/41 (32%): ≥ 7mm < 10mm 7/25 (28%) in >10mm No significant difference	ETs in CC-IVF should not be an exclusion criterion Comparable pregnancy rates	Old study No p-values recorded

LH=Luteinizing hormone, ET=Embryo transfer, E₂=Oestradiol, TDS=three times a day, NC=Natural cycle, CC=Clomiphene Citrate, CPR=Clinical pregnancy rate, VEGF=Vascular endothelial growth factor, FF=Follicular fluid, OC=Oral contraceptive, hMG= human menopausal gonadotropin, PR = Pregnancy rates

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Awonuga <i>et al.</i> 1997 [65]	Retrospective	11 non responders 20 poor responders treated with CC (low dosage) + IVF All the patients were previously treated with hMG +GnRH agonist	n= 11 received CC n=20 received CC	CPR per oocyte collections Non-responders, (9.1%) Poor responders (10%) CPR in previous long agonist protocol 11.9%. No significant difference	There is no significant benefit/advantage of using long protocol in poor responders.	The numbers were too small to generate a sound opinion.
Branigan et al.2000 [29]	Prospective Cohort	32 women with tubal or pelvic adhesive disease, normal ovulating cycles, under the age of 40. Received CC 100mg from cycle day3, for 8 days.	Two months ovarian hypothalamic suppression with OC (Desongen) for LH suppression.	No LH surges occurred. Mean mature oocytes retrieved: 3.2 90% fertilisation rates Mean embryos transferred: 2.5 CPR: 32.8% (21/64) per retrieval	Protocol is a low cost and low risk alternative to conventional IVF with comparable PRs.	The use of OCP for LH suppression is a cheap acceptable strategy in IVF

LH=Luteinizing hormone, ET=Embryo transfer, E₂=Oestradiol, TDS=three times a day, NC=Natural cycle, CC=Clomiphene Citrate, CPR=Clinical pregnancy rate, VEGF=Vascular endothelial growth factor, FF=Follicular fluid, OCP=Oral contraceptive pill, hMG= human menopausal gonadotropin

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Tokuyama <i>et al.</i> 2002 [31]	Prospective Comparative study	38 patients undergoing IVF-ET, divided into 3 groups: determine VEGF in FF Group 1: hMG (n=19) Group 2: CC (n=10) Group 3: natural cycles (n=9)	hMG cycle and CC treatment	Group 1 show lower VEGF in FF than group 2 or 3. Group 1 had higher number of oocytes harvested. However the results were not significant.	VEGF concentration in FF correlates with number of follicles irrespective of the ovulation induction protocol.	Role of endocrine markers in IVF still needs to be emphasised in selected patients Their role cannot be recommended routinely
Ragni <i>et al.</i> 2012 [28]	RCT non-inferiority trial	304 women with compromised ovarian reserve based on day 3 FSH>12IU/ml on two occasions, or previous poor response (< 3 oocytes) to hyper stimulation n=148 to CC n=156 to short GnRH α protocol with high doses of gonadotropins	CC 150mg day 3-7	The delivery rate per started cycle were: CC: n=5/148 (3%) GnRH α : n = 7/156 (5%) P=0.77 No significant difference No side effects were observed.	In women with compromised ovarian reserve, ovarian stimulation with CC or high dose gonadotropins led to similar pregnancy chance but CC is less expensive.	Premature cessation of the study reduced the sample size and affected the power negatively.

LH=Luteinizing hormone, ET=Embryo transfer, E₂=Oestradiol, TDS=three times a day, NC=Natural cycle, CC=Clomiphene Citrate, CPR=Clinical pregnancy rate, VEGF=Vascular endothelial growth factor, FF=Follicular fluid, OC=Oral contraceptive, hMG= human menopausal gonadotropin

Table 2, Part B: Clomiphene in combination with gonadotropins with/without GnRH antagonists

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Williams et al. 2002 [14]	Retrospective case-controlled study	55 patients underwent IVF using CC 100mg day 3-7 then 150IU of Gonadotropin from cycle day 9 until day of hCG. Embryo transfer on day 3 after retrieval. Compared to 55 patients undergoing standard GnRH α long protocol and ET on day 3.	CC + Gonadotropin stimulation in normal responders. No antagonists.	Less medication (5.7 \pm 4.2 v/s 25.0 \pm 7.5 ampoules, P<0.5) Less mature oocytes (4.8 \pm 2.6 v/s 16.2 \pm 7.5, P<0.5) Fewer ET (2.9 \pm 1.1 v/s 3.5 \pm 0.9 P<0.5) CPR were equivalent in both groups; (16/43, 37% vs. 21/51, 41%) [P=0.85] OPR also similar; 13/43, 30% v/s 17/51, 33% P=0.92 16% cycles cancellation due to poor follicular growth in CC v/s 7% in GnRH α . Cost of medication: 45% less in CC/Gonadotropin group.	Equivalent CPR/OPR 45% reduction in cost to patient \uparrow risk of cycle cancellations Little cryopreservation	A simple and cheap alternative.

CC= Clomiphene Citrate, IU= International Units, ET= embryo transfer, COC= combined oral contraceptive, CPR= clinical pregnancy rate, OPR= ongoing pregnancy rate, LBR = live birth rate, CD= menstrual cycle day, recFSH & rFSH= recombinant follicle stimulating hormone, recLH & rLH=recombinant luteinizing hormone, RCT= randomized controlled trial, EP= European pound, mcg= microgram

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Weigert et al. 2002 [43]	Prospective randomised study	294 infertile women undergoing IVF-ET 154 cycles stimulated with CC + rec FSH + rec LH and 140 cycles with long GnRHa + rec FSH.	CC:100mg CD1 - 5 rec FSH: rec LH 3:1 (225:75IU) every alternative day. Prednisone 7.5mg × 1 month. All patients pre- treated with COC for 18-26 days. No antagonists.	Pregnancy rate per ET 42.9% in CC group vs. 36.6% in long GnRHa protocol. No significant difference Cancellation rate were similar (16.9% vs. 15.7%) [p=0.3] OHSS higher in long GnRHa protocol group (10% vs. 3% in CC group) [p=0.02] Significant	Comparable pregnancy outcome Less gonadotropin used. Significantly reduced risk of OHSS in the non agonist group.	Good outcomes.

CC= Clomiphene Citrate, IU= International Units, ET= embryo transfer, COC= combined oral contraceptive, CPR= clinical pregnancy rate, OPR= ongoing pregnancy rate, LBR = live birth rate, CD= menstrual cycle day, recFSH & rFSH= recombinant follicle stimulating hormone, recLH & rLH=recombinant luteinizing hormone, RCT= randomized controlled trial, EP= European pound, mcg= microgram

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Tavaniotou et al. 2002 [15]	Retrospective analysis	40 patients enrolled Group I: 20 patient stimulated with CC + Gonadotropin + Cetrorelix 0.25mg Group II: 20 patients stimulated with Gonadotropin + 0.25mg Cetrorelix Evaluating LH in follicular + luteal phase during IVF cycle.	CC 100mg/d, CD2-6 HMG or rFSH from day 4 Cetrorelix 0.25mg from day 7.	LH levels were significantly higher during follicular + luteal phases in CC stimulated cycles. (5.2 ± 4.2 IU vs. 1.8 ± 1.4 IU in non CC group, P<0.001)	LH concentrations are significantly higher in the follicular + luteal phase of CC stimulated cycles despite antagonist administration. Simply managing a dose adjustment is necessary to suppress LH surge.	Data very heterogeneous using CC with rFSH or HMG Although the study expressed higher levels of LH in follicular + luteal phase, no comment on clinical pregnancy rates.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Engel et al. 2002 [16]	Prospective non- randomized trial	Evaluate the possibility of using CC in combination with gonadotropin in antagonist protocol. Specially to evaluate the equivalence of rFSH and HMG with regards to the rate of HCG administration. 107 patients HMG group n=54, rFSH group n=53 between June 1997 and Oct 1999.	Protocol I: CC 100mg from CD2/3 for 7 days rFSH or HMG from CD 6 (225IU) Cetorelix 0.25mg from CD6 until day of HCG All ICSI cycles Protocol II: CC 100mg x 5/7 only Protocol III: CC 100mg x 5/7 plus FSH or HMG on CD 6 (150IU) Protocol IV: CC 100mg x 5/7 plus FSH or HMG from CD 3 (150IU)	Slightly more oocytes but not significant difference with HMG (7.2 ± 5.31 vs. 5.5 ± 3.75) Similar number embryos obtained in both HMG and rFSH group (3.64 ± 2.43 vs. 3.38 vs 1.87) CPR were comparable; (25.9% HMG vs. 13.8% rFSH) Overall LH Surge rate was 21.5% Protocol I - 27.3% Protocol II - 25% Protocol III – 11% Protocol IV -27.5 % The rate was always higher in rFSH group (26.4% rFSH vs. 16.7% HMG) explaining slightly reduced PR.	Increase risk of premature LH Suggesting caution in the use of CC soft stimulation protocols. No significant difference in CPR	Inconsistency in the protocol

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Mansour et al.2003 [55]	Comparative study	189 couples, first ICSI treatment. Group 1 (n=33): CC 150mg/d CD 2-6 HMG 150 IU, days 6-10 Cetrorelix 0.25mg/d when leading follicle is > 16mm in the absence of LH surge. Group II (n=156): 0.1 mg Decapeptyl a day + HMG	CC + HMG + Cetrorelix	CPR: 8/33 (24%) in CC group vs. 92/156 (59%) in long protocol (P=0.019) Statistically significant Cost of medication: 1110 ± 492 EP in CC vs. 1928 ± 456 in long protocol. Total cost per pregnancy: 19653 EP in CC vs. 10047 EP in GnRH _a .	CC+ HMG + antagonist protocol is not cost effective and should not be recommended.	Very small numbers in CC group

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Hwang JL et al. 2003 [56]	Observational study	40 women undergoing ICSI for male infertility. CC 100mg from day 3-7 + HMG 150 IU days 4, 6, 8 and from day 9 adjusted according to follicular response. Cetorelix 2.5mg given once follicle of 14mm is present. If no hCG given after 4 days of Cetorelix then 0.25mg were given daily until day of HCG. Evaluate the efficacy to suppress premature LH surge	CC + HMG + Cetorelix initial high dosage (2.5mg) plus added small dosage (0.25mg) if necessary	4/40 (10%) needed additional 0.25mg Cetorelix. Single dose 2.5mg Cetorelix effectively suppressed the LH surge for 4 days in all patients. Premature LH surge did not occur in any patients. The CPR & OPR were 16/40(40%) and 14/40 (35%) respectively.	LH was successfully prevented by Cetorelix 2.5mg once off dosage.	Single high dosage of Cetorelix appears to be effective for LH suppression versus multiple small dosages

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Tavaniotou et al. 2003 [62]	Prospective trial	46 patients enrolled Group 1: 18 sequentially stimulated CC + gonadotropin + Cetorelix 0.25mg. Group II: 28 Simultaneous stimulation with CC + gonadotropin + Cetorelix 0.25mg Evaluate for premature LH surge and its impact on pregnancy outcomes.	Sequential CC + gonadotropin + Cetorelix versus Simultaneous CC + gonadotropin + Cetorelix	Cycle cancellation rates: 22% in sequential protocol vs. 7% in simultaneous group (significant difference) CPR per ET: 18.1% in sequential group vs. 29.1% in simultaneous group. (not significant) Premature LH surge was 11.1% in sequential vs. 28.5% in simultaneous group. Pregnancy rates were 12.5% with LH surge vs. 29.6% in patient without LH surge.	Sequential CC/HMG is not recommended for CC/HMG/antagonist cycles. Cetorelix 0.25mg was associated with high LH surge. Premature LH surges were associated with adverse treatment outcome.	Type of CC/HMG regimen seems to be important for prevention of premature LH surge. Trials will be needed to evaluate this effect in a non-antagonist protocol.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Engel et al. 2003 [54]	Prospective randomized feasibility study	10 patients enrolled. n=5: CC + HMG + Cetrorelix 3mg single dose. n=5: CC + rFSH + Cetrorelix 3mg single dose. Evaluate the efficiency to suppress LH.	CC + HMG + Cetrorelix and CC + FSH + Cetrorelix.	No premature LH surge (LH > 10IU/ml) or (progesterone > 1ng/ml) occurred. Overall take home baby rate of 30%. No significant difference in the outcomes.	Single dose Cetrorelix 3mg is effective in preventing premature LH surge.	Study involved small numbers

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Fiedler et al. 2003 [60]	Retrospective data analysis	1354 patients treated with CC + gonadotropin + antagonist between Jan 1998 → Dec. 2001. CC: 100mg CD 5-9, HMG or rFSH 150 IU from day 9 until day of HCG. Cetrorelix 0.25mg daily from day 10 until day of HCG. 4704 patients treated with long GnRH agonist protocol. No routine measurement of LH.	CC + HMG or rFSH + antagonist versus Long GnRH agonist protocol.	LBR per cycle: 21.3% GnRHa vs. 19.5% CC CPR per cycle: 1594/4704 (33.9%) long protocol vs. 424/1354 (31,3%) CC Miscarriage rate: 21.6% long protocol vs. 22.9% CC protocol. No significant difference between two groups	CC + gonadotropins + antagonists is an ideal protocol in selected group of patients	Comparable outcomes in both regimens P values not recorded

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Lin YH et al. 2007 [64]	Prospective observational study	50 patients who had excessive ovarian response required coasting but failed to conceive. CC: 100mg CD 3-7 HMG: 2-3 ampoules on day 4,6,8 then daily from day 9 Cetrorelix 2.5mg when leading follicle >14mm. If no HCG after 4 days, 0.25 mg was added daily until day of HCG. Evaluate the incidence of OHSS, E2 and need for coasting	CC + HMG + Cetrorelix treatment versus Long protocol GnRHa	Coasting 50/50 (100%) vs. 4/50 (8%) P<0.05 No premature LH surge in CC HMG group Cycles cancelled: 8/50 (22%) GnRH vs. 1/50 (2%) CC [P<0.05] OHSS: 9/50 (18%) GnRH vs. 1/50 (2%) CC [P<0.05] Severe OHSS: 1/9 (2%) v/s 0% (NS) CPR per fresh ET per cycle: 3/50 (6%) v/s 21/50 (42%) P<0.05(significant) Significant higher cancellation rates & OHSS rates with GnRH	CC + HMG + Antagonist ideal alternative for patients with previous hyper response.	Ideal protocol to prevent OHSS

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Lin YH et al. 2007 [63]	Prospective observational study	113 couples with male factor infertility undergoing ICSI. 106 cycles analysed. CC 100mg CD 3-7 + HMG day 4, 6, 8, then daily from day 9. Cetorelix 2.5mg once off when the leading follicle is ≥ 14 mm. Evaluate oestradiol patterns throughout the cycles.	CC + HMG + Cetorelix	Serum oestradiol rose in 48 cycles (45,3%) after Cetorelix administration. It plateaued in 26 cycles (24.5%) + dropped in 32 cycles (30.2%) CRP: 37.5% in E2 rise vs. 42.3% E2 plateau vs. 37.5% in E2 drop.No significant difference. No correlation between LH and E2 levels. All patients had ET.	Oestradiol patterns after Cetorelix injection show no correlation with clinical outcome + ovarian reserve. Falling oestradiol is not associated with adverse outcome Similar pregnancy rates were observed.	The role of oestradiol in IVF cycle continued to be questioned and defined.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Yanaihara et al. 2008 [58]	Retrospective analysis	308 IVF cycles for IVF via mild ovarian stimulation: CC 100mg day 3-7, rFSH daily from day 5 daily/or HMG in repeat every other day. Cetrorelix daily 0.25mg when dominant follicle > 14mm LH evaluated at the time of antagonist and at the time of HCG injection	CC + rFSH + Cetrorelix	50/308 (16%) LH level dropped less than one- third and the control LH level were within 1/3. CPR: 18% in the LH levels < 1/3 vs 39% in the control group. IPR: 18% in the LH levels < 1/3 vs 26% in the control group.	LH levels below 1/3 at the time of HCG, is associated with significantly lower IPR and CPR.	Caution for severe LH suppression with high dose Cetrorelix

CC= Clomiphene Citrate, IU= International Units, ET= embryo transfer, COC= combined oral contraceptive, CPR= clinical pregnancy rate, OPR= ongoing pregnancy rate, LBR = live birth rate, CD= menstrual cycle day, recFSH & rFSH= recombinant follicle stimulating hormone, recLH & rLH=recombinant luteinizing hormone, RCT= randomized controlled trial, EP= European pound, mcg= microgram

Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Noorashikin et al. 2008 [48]	Prospective non randomized controlled study	95 first cycles ICSI patients. n=54: low dose CC 100-150mg CD2-6 rFSH 75-150 IU No Antagonists n=41 Standard dose gonadotropin + antagonist protocol rFSH 225-450IU from CD2 Cetrorelix 0.25mg/d when leading follicle is 13 mm until HCG trigger	CC + low dose rFSH only No antagonists versus Standard dose rFSH + Antagonist protocol.	Fewer oocytes in LS vs standard (P<0.0005) Fewer embryos in LS vs standard (P<0.0005) CPR per transfer: 43,2% LS vs 50% Standard (not significant) More freezing cycles in standard than in LS (P<0.001)	Comparable CPR Less medication in LS Lower cost in LS	Acceptable CPR without antagonists' administration. No comment on LH surge in the non-antagonist group.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Karimzadeh et al. 2010 [23]	Prospective RCT	243 enrolled between Jan 2008-Dec 2008 but only 200 were analysed per group. Group B: CC 100 CD 3-7 rFSH 75IU daily from CD5 GnRH antagonist Cetrorelix 0.25mg/d when dominant follicle is ≥ 12 mm Added HMG 75IU to rFSH (= 150 IU) from day 8 Group A : Buserelin from previous menstrual cycle day 21 day (long protocol) + rFSH 150-225IU daily. Main outcome was OPR.	Group B: CC + gonadotropin + antagonists versus Group A: Buserelin long agonist protocol.	Significantly higher. Number of oocytes in Group A vs Group B (9 ± 2.2 vs 5.42 ± 1.5 , $P=0.00$) No reported cases of OHSS in CC group [6(6%) v/s 0, $p=0.02$] 4 cycles (4%) cancelled in CC group v/s none in Buserelin group. Significantly more gonadotropin used in Group A vs Group B (22 ± 3.6 v/s 12.1 ± 4.3 , $P=0.00$) OPR per transfer: 26.5% in Group A vs 33.3% in Group B (NS) No difference in endometrial thickness ($P=0.41$)	CC + gonadotropin +antagonist protocol is as effective as conventional protocols.	No comment on LH surge in the CC group Noted cancellation rates in CC but comparable and favourable outcomes.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Aleyamma et al. 2011 [49]	Retrospective analysis	143 women evaluated: CC: 50 mg daily from CD2 until the day of HCG. Intermittent HMG 150IU from CD5 on alternative days Cycles monitored by USS only.	CC + HMG protocol. No antagonists	104/143 underwent ET (73%) and 27% cycles cancellation rate LBR per cycle = 14% (20/143) LBR per transfer = 19% No cases of OHSS	Authors felt that the LBR were acceptable at a reasonable cost. It is an option for patient who cannot afford conventional IVF.	At least LH monitoring should be considered in minimal stimulation using CC.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Jovanovic et al. 2011 [26]	Retrospective study	4859 poor responders enrolled between 2005-2009 but 125 cycles involving 48 patients were analysed. Group 1: Gonadotropin 450 – 600 IU + CC 100mg CD 2-6 + Cetorelix 0.25mg when leading follicle \geq 13mm. Group II: Gonadotropin 450-600IU + Cetorelix 0.25mg as above 29 patients involving 85 cycles were also analysed. Group I: CC + gonadotropins as above Group II: Letrozole 5mg CD 2-6 + gonadotropins	A: CC + high dose gonadotropin versus High dose gonadotropin alone. B: CC + gonadotropin versus Letrozole (LZ) + gonadotropin	A: CPR: 2/55(4%) in Non-CC vs 5/60(8%) CC group (Not significant) Less cancellation cycles in CC group ($p < 0.05$) Significant B: CPR: 0% CC vs 2/45 (4%) LZ group Similar endometrial thickness (8.2 ± 0.4 mm vs. 8.2 ± 0.4 mm) No significant difference in cycle cancellations	CC as an adjunct to high dose gonadotropins in poor responders is beneficial. LZ group was associated with increased oocytes at retrieval but overall not superior to CC as an adjunct.	Data is very heterogeneous and the numbers were small.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Gibreel et al. 2012 [19]	Systematic review & meta-analysis	2536 participants (14 randomised studies) 6 studies: CC + gonadotropin vs long gonadotropin GnRHa protocol. 2 studies: CC + gonadotropin(Gn) vs short protocol GnRHa 1 trial: 3 arm study: CC + Gn vs. GnRHa long vs. GnRHa short 1 trial: CC + Gn + mid cycle antagonist (Ant) protocol 4 trials: CC + Gn + mid cycle Ant vs. long GnRHa	CC ± Gn ± mid cycle antagonist versus Long and short agonist GnRHa protocol.	Similar outcomes regarding LBR or CPR in CC + Gn ± mid cycle antagonist vs. long or short GnRHa LBR (5 RCTs, 1079 women; OR 0.93, CI 0.69 – 1.24) CPR (11 RCTs, 1864 women; OR 1.07, CI 0.85 – 1.33) Significant reduction in OHSS: (5RCTs, 1559 women; OR 0.23, CI 0.10 – 0.52)	No significant difference in outcomes in terms of LBR or CPR between CC + Gn + Ant vs long agonist protocol. Significant reduction of OHSS.	Review included old studies No cost comparison in the analysis. Review included studies by Ashrafi [42], Fiedler [60], Karimzadeh [23] and Weigert [43].

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Figueiredo et al. 2013 [24]	Systematic review and meta-analysis	CC + Gn + Ant vs. GnRHa protocol 7 trials (702) participants	CC + Gn + Ant vs long agonist protocol	LBR: No significant difference between two protocols (55/182, 30.2% CC vs. 47/181. 26.0%, non-CC P=0.26) CPR : (98/346, 28.3% CC vs. 84/356, 23.6% non-CC, P=0.12) No difference in other outcomes such as endometrial thickness and number of oocytes retrieved. Significant reduction in OHSS (0.5% vs. 4.1%, P=0.01)	CC + Gn + Ant protocol is associated with reduced amount of medication and significant reduction in OHSS risk but no effect on LBR and CPR.	Similar findings as the Cochrane review. The review included the following studies: Karimzadeh [23] and Karimzadeh [59]

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Ashrafi et al. 2005 [42]	Randomised prospective trial	154 Poor responders enrolled between June 2003 – July 2004 Group 1: n=45, HMG 150 IU from CD3 Group 2: n=52, GnRHa long protocol + HMG 225 IU/d Group 3: n=34 100mg CC CD3-7 + HMG 150IU from CD6 Evaluate LH surge, number of oocytes, and cancellation rates.	CC + Gn No antagonists	LH was higher in all groups except in the long GnRHa protocol (30.5% in HMG vs. 28% in CC vs. 0% GnRHa, P=0.004) Significant Similar cancellation rate (P=0.537) NS More ampoules used in GnRHa (P=0.0001) Significant	Similar cancellation rates and mainly due to poor follicular response and premature LH surge.	Risk of premature LH surge noted in both CC and HMG cycles.

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Study	Design	Study	Intervention	Outcomes	Conclusion	Comment
Karimzadeh et al. 2011 [59]	Randomised controlled trial	159 poor responders enrolled between March 2008 – May 2010 (women \geq 38yrs, previous IVF with \leq 3 oocytes retrieved after conventional stimulation \pm E2 \leq 500pg/ml on the day of HCG) All received COC x 21 days Group 1: n=79, CC, CD 3-7. rFSH/HMG 225 IU from day 5 daily. Daily Ganirelix 0.25 mg daily from leading follicle of \geq 14mm Group II: n=80, Buserelin 56/mcg twice daily from cycle day 2, rFSH/HMG for 225-300 IU/d from CD2	CC + Gn + Ant versus Buserelin + Gn protocol	More gonadotropin in microflare protocol (P=0.000) More days of stimulation in microflare (P = 0.003) Endometrial thickness higher in mild / CC protocol (P = 0.001) More oocytes + mature oocytes in microflare protocol (P=0.005) Similar cycle cancellation in both groups. CPR/cycle: 24.10% vs. 16.20%, slightly higher in CC group but NS. CPR/transfer 31.10% vs. 21%, NS.	Reassuring CPR with mild CC protocol Fewer days of stimulation in CC protocol. Less stressful to patients.	GnRHa appears not to be effective and ideal for poor responders.

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DISCUSSION

The ongoing need to simplify ART, particularly controlled ovarian stimulation, should remain the fundamental purpose and goal for all involved with the care of infertile couples. It is simply to make it patient friendly, less stressful and to reduce the cost of medication. However, this must be guarded against pregnancy outcomes.

Currently the long GnRH agonist protocol is still regarded as the gold standard regimen for ovarian stimulation in ART [1]. However, the GnRH antagonist protocols are slowly becoming the routine in most units. Both of these protocols are generally expensive, using large amounts of gonadotropins [24].

This therefore emphasises the need to continue to explore the mild ovarian stimulation protocols in ART. CC protocols should be appraised and carefully be used on an individual basis.

Clomiphene Citrate alone

We have reviewed a total of seven studies that reported the outcomes of ART in CC only IVF/ICSI cycles (Table 1, Part A). There were two RCTs in which one study by McDougall *et al.* [27] compared CC cycle and NC IVF outcomes. The author reported significantly higher CPR in CC (18%) vs NC (0%) [27]. Another trial by Ragni *et al.* [28] evaluated 304 women with compromised ovarian reserve. Participants were allocated to receive CC only (n=148) or short GnRH agonist treatment with high doses of gonadotropins (n=156). The delivery rates per started cycle were comparable, CC (3%) vs GnRHa (5%) pvalue = 0.77 [28]. However the CC regimen was much cheaper than the agonist protocol. Three prospective studies by Branigan *et al.* [29], Gentry *et al.* [30] and Tokuyama *et al.* [31] reported reassuring results. CPR of 32.8% was observed in a study by Branigan *et al.* involving 32 women with normal ovulatory cycles treated with CC alone following two months of ovarian hypothalamic suppression with OCP [29]. Gentry *et al.* further showed comparable and acceptable CPR at different endometrial thickness measurements, (20%) : >4mm <7mm, (32%): > 7mm< 10mm and (28%) in >10mm in women treated with CC only [30]. The final outcome of ART treatment is to achieve a successful pregnancy and a singleton live birth [32]. In the

early days of IVF, the outcomes were reported to be generally poor due to a combination of factors [33]. In an attempt to improve pregnancy outcomes, quality assurance in the laboratory has improved, skills development and training of scientists and clinicians has also improved and ovarian stimulation protocols have changed and continue to be modified on individual bases. Higher numbers of embryos were also transferred as a strategy to increase pregnancy rates, at the risk of multiple pregnancies with subsequent financial burden on individual patients and the health system as a whole [34,35]. The suggestion of mild ovarian stimulation together with single embryo transfer to lower the complication risks of IVF/ICSI treatment became widely accepted in countries in which IVF is government subsidised [34,36].

Mild ovarian stimulation protocol is defined as ovarian stimulation using oral agents alone or in combination with gonadotropins with or without mid-cycle GnRH antagonist, with the aim of retrieving ≤ 7 oocytes per cycle [4]. Clinical pregnancy rates in IVF cycles of normal responders using CC alone could vary from 16% per started cycle to 34% per embryo transfer. [29, 30, 37, 38].

In studies that reported low pregnancy rates, endometrial thickness of (<7 mm) and failure to support the luteal phase were postulated reasons for the poor outcomes. There are also reports of pregnancy rates over 30% with endometrial thickness of >7 mm, adequate suppression of premature LH surge and luteal phase support independently influencing the outcomes [29, 30, 37]. In comparison with natural cycle IVF, the CC alone stimulated cycle had $>90\%$ chance of oocyte retrieval and embryo transfer [29, 38]. But again, the risk of premature LH surge in CC stimulated cycles associated with poor oocyte quality and as a result high pregnancy wastage remains a concern [39].

In women with poor ovarian response or compromised ovarian reserve, the use of CC alone in IVF has been associated with pregnancy rates of 5-10% [28, 40]. Although the rates were low, they were comparable to those of women treated with conventional long GnRH agonist protocol and large amounts of gonadotropins [40]. Therefore, CC alone IVF may be considered an option in older women with depleted ovarian reserve. It also appears to be better than natural cycle IVF. It is interesting to note that the anti-oestrogenic adverse effects of CC were not reported as the major concern in these

studies. More so, CC alone IVF could present another option in very limited resource settings.

The limitations of the studies we reviewed are that most trials were old, sample sizes were small and the data was heterogeneous with different outcomes. Future studies should evaluate the role of aromatase inhibitors such as Letrozole [41] in IVF with regard to pregnancy rates, premature LH surge and endometrial thickness.

Clomiphene Citrate plus gonadotropins without gonadotropin releasing hormone antagonists

We have reviewed a total of five studies that reported the outcomes of ART in CC plus gonadotropins without GnRH antagonist IVF/ICSI cycles (Table 2, Part B). There were two RCT's, one by Ashrafi et al [42] that evaluated 154 women with poor ovarian response, group 1 (n= 45, HMG only), group 2 (n= 52, GnRHa long protocol + HMG) and group 3 (n=34, CC + HMG). The results showed significantly higher LH surge in HMG group (30.5%) vs CC/HMG (28%) vs GnRHa (0%) pvalue=0.004. Significantly more ampoules of gonadotropins were required in the GnRHa group (pvalue=0.0001) [42]. However, the authors reported similar cancellation rates in all groups HMG (38.8%) vs GnRHa (50.1%) vs CC/HMG (45.5%) p=0.537 [42]. Similar findings of significantly high cancellation rates were reported by Gibreel *et al.* in a systematic review and meta-analysis [19]. Another trial by Weigert *et al.* [43] randomised 154 infertile women to receive CC + recFSH + recLH and 140 infertile women to receive long protocol GnRHa + recFSH. Pregnancy rates per ET were reported as 42.9% in the CC group vs 36.6% in the GnRHa group and not significant [43]. The cancellation rates were similar in both groups, 16.9% CC vs 15.7% GnRHa and also not significant [43]. However the risk of OHSS was significantly higher in the GnRHa group (10%) vs CC group (3%) pvalue=0.02 [43].

Gonadotropin preparations have been commercially available since the 1960s and since 1978 following the birth of the first IVF baby they have been used increasingly in ART such as IVF or ICSI [44]. Gonadotropins may be used alone or in combination with oral agents. The aim of adding oral agents such as CC to gonadotropins is to

increase the number of oocytes for fertilization and to use less expensive gonadotropins.

A retrospective study by Williams *et al.* [14] reported comparable clinical pregnancy rates per transfer of (37% vs 41% $pvalue=0.85$) in minimal stimulation vs long GnRHa protocol respectively. In a sub-analysis of data comparing GnRH antagonist, Ganirelix (n=10) vs non Ganirelix group (n=10), there was no statistically significant difference in the number of oocytes retrieved (4.9 ± 3.1 vs 3.1 ± 1.0) and the number of embryos transferred (2.5 ± 1.1 vs 2.8 ± 1.1) [14]. There were no reported cases of premature LH surge in this study and the cost of medication was 45% less in the CC and gonadotropin group [14].

Seqawa *et al.* in a study of 3654 infertile women showed equal CPR of 34.1% in CC + 150 IU of hMG versus 34.2% in CC + 75IU of hMG on alternate days [45]. Another retrospective analysis of over 19000 IVF cycles from a single unit reported impressive pregnancy rates of 43.6% using CC 50mg for five days plus 50IU hMG from cycle day 8 [46]. In a pilot study by Garzo *et al.*, embryo quality was found to be significantly better in a low stimulation protocol [47]. Noorashikin *et al.* in a prospective non-randomized trial comparing (n=54) CC + delayed rFSH without antagonist versus conventional rFSH + GnRH antagonist, Cetrorelix (n=41) found no statistically significant difference in CPR between two groups (43.2% in CC vs. 50% in antagonist group) [48]. There were more freezing cycles in the antagonist group ($P < 0.001$) but the total treatment cost was also higher in the antagonist group (USD 13 200 LS vs. USD 24 900 antagonist) [48].

The reported high rates of cycle cancellation [42] [43] [49] are the main reason behind the notion and promotion of short mid-cycle introduction of GnRH antagonists in IVF/ICSI treatment.

Clomiphene Citrate plus Gonadotropins with mid-cycle gonadotropin releasing hormone antagonists

We have reviewed a total of 15 studies that reported the outcomes of ART in CC plus gonadotropins with mid-cycle GnRH antagonist IVF/ICSI cycles (Table 2, Part B). The findings of two systematic reviews and meta-analysis by Gibreel *et al.* [19] and Figueiredo *et al.* [24] were similar. Gibreel *et al.* [19] analysed all RCT's that compared CC, alone or in combination with gonadotropins, with conventional gonadotropin cycle (GnRH agonist or antagonist). Fourteen RCTs involving 2536 participants showed comparable outcomes between two regimens. LBR from 5 RCTs, n=1079 participants did not show any statistical difference (OR 0.93, CI 0.69-1.24) [19]. Other outcome measures such as OPR and CPR did not show any statistical difference either [19]. In five studies (n=1559 participants) there was a statistically significant decrease in the incidence of OHSS in CC protocol (OR 0.23, CI 0.10-0.52) [19]. In a second review by Figueiredo *et al.*, [24], seven RCT's (n=702 participants) that compared CC + gonadotropins + GnRH antagonists with gonadotropins + GnRH agonists or antagonists were analysed. LBR were reported by two studies and no significant difference was observed (RR 1.16; CI 0.84-1.62) [24]. CPR from all seven studies did not show any significant difference either (RR 1.22, CI 0.95-1.56) [24]. There was significant reduction in the risk of OHSS in the CC group with Peto OR 0.20, CI 0.06-0.69 [24].

The GnRH antagonist was originally developed as a non-steroid contraceptive drug [50] but eventually became useful and beneficial in assisted reproduction. Mid-cycle use of GnRH antagonist in ART results in immediate suppression of pituitary gonadotrophins for a period of 8 hours with rapid recovery of normal secretion of endogenous LH and FSH [50, 51]. The use of a GnRH antagonist in a standard IVF when compared with a GnRH agonist protocol have shown no significant difference in probability of live birth and ongoing pregnancy rates [17, 19, 52, 53]. There was significantly lower incidence of OHSS in the GnRH antagonist group ($P < 0.00001$) [53].

Mid-cycle administration of a GnRH antagonist in mild ovarian stimulation has been associated with conflicting results, with some authors reporting unacceptably high levels of premature LH surge ($LH > 10IU$) before HCG trigger [15,16]. In both studies,

the dosage of the GnRH antagonist used was a fixed regimen of 0.25mg daily from cycle day 6 or 7. A follow-up randomised feasibility study by Engel *et al.*, using a higher single dose of GnRH antagonist, Cetrorelix 3mg, reported no cases of premature LH surge with a take home baby rate of 30% [54] – therefore concluding that a single dose of 3mg is effective in the prevention of premature LH surge.

Mansour *et al.* reported that a CC + hMG + mid-cycle antagonist protocol is not cost effective and should not be recommended [55]. However, more studies further reported effective suppression of premature LH surge with a 2.5mg single dose of Cetrorelix, adding 0.25mg daily only if no HCG is given after 4 days since the initial Cetrorelix administration [56, 57]. Yanaihara *et al.*, however, cautioned that severe drop in LH levels below a third (1/3) of the baseline may result lower pregnancy rates (18%) in low LH <1/3 versus (39%) in the control [58].

Subsequent trials reported a comparable clinical pregnancy rate with no significant difference between a CC/hMG/antagonists protocol and a long GnRH agonist protocol [23, 57]. Similar findings were also reported by Karimzadeh *et al.* in a study of poor responders, with CPR per started cycle of (24% in CC vs 16% in Buserelin) [59]. More studies reported LBR of 19.5% in CC vs 21.3% GnRHa and 36% in CC vs 35% in GnRHa [57, 60].

The antagonist protocol is still relatively unaffordable, particularly in a limited resources setting, therefore simpler and cheaper methods to lower the risk of premature LH surge amongst other adverse events of CC still need to be explored. Kawachiya *et al.*, in a study of 543 cycles using CC 50mg from cycle day 3 until the day of HCG or GnRH agonist trigger, reported lower rates of premature LH surge (5%), therefore suggesting that CC was sufficiently effective in suppressing LH surge in the minimal stimulation IVF protocol [61]. In another study, Branigan *et al.* also reported no cases of premature LH surge with two months' pre-treatment of an oral contraceptive pill prior to stimulation with CC 100mg from cycle day 3 for 8 days [29].

Tavaniotou *et al.* proposed the simultaneous use of CC and gonadotropins as opposed to the sequential protocol (begin stimulation with CC and add gonadotropins later in

the cycle) since the former was associated with lower risks of premature LH surge (7% versus 22%) [62].

CONCLUSION

The current available evidence suggests CC + gonadotropin + mid-cycle antagonist protocol as an effective protocol with comparable live birth rates and ongoing pregnancy rates versus long GnRH agonists protocol. In addition, it is associated with reduced risk of OHSS and a lesser number of gonadotropins required. CC + gonadotropins without antagonists also appear to be a feasible protocol in well selected groups of patients, young and normal endocrine profile. The risk of premature LH surge may be minimised by pre-treatment with an oral contraceptive pill or prolonged use of CC during stimulation or simultaneous use of CC and gonadotropins. However, all these strategies still need to be evaluated rigorously in large trials. The future of CC alone in IVF is rather doubtful, especially in the era of gonadotropins with or without GnRH antagonist protocols. Perhaps in a selected group of patients, young women with normal endocrine profile and women with compromised ovarian reserve or previous poor response to conventional stimulation, the CC alone protocol may present an alternative with fair results and at a low cost.

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CHAPTER 3: A SIMPLE METHOD OF EXTENDED 8-DAY COURSE OF CLOMIPHENE CITRATE VERSUS 5-DAY COURSE IN AN ATTEMPT TO SUPPRESS PREMATURE LUTEINIZING HORMONE SURGE IN AN ASSISTED REPRODUCTIVE TECHNOLOGY PROGRAMME: A RANDOMIZED CONTROLLED TRIAL

SYNOPSIS

Objective: To determine whether the use of a prolonged eight-day course of CC versus the standard use of five days is an effective method to prevent premature LH surge in an ART programme.

Design: Prospective randomised controlled study.

Setting: Tygerberg Academic Hospital, Reproductive Medicine Unit.

Population: Couples attending fertility clinic for IVF/ICSI treatment for female and male infertility problems.

Methods: Eligible participants were randomised into one of two treatment groups using a simple randomization schedule assigned via numbered sealed envelope following patient assessment. In Group A, the patients were stimulated with CC 100mg for 5 days from cycle day 3 to 7 plus hMG 150-225IU that was added on cycle days 4, 6, 8 and/or 10. In Group B patients were stimulated with CC 100mg for 8 days from cycle day 3 – 10 plus hMG that was added on cycle days 4, 6, 8 and/or 10. Cycles were monitored with ultrasound and urinary LH tests. No endocrine blood tests were performed. hCG was administered when there was a dominant follicle of ≥ 18 mm, followed by oocyte retrieval 34-36hrs later. The luteal phase was supported with vaginal progesterone.

Main outcome measures: The rates of premature LH surge, cycle cancellation rates, live birth and clinical pregnancy rates.

Results: Two hundred and thirty eight (238) patients were enrolled and randomized and a total of 227 were analysed. The percentage risk of premature LH surge was similar in both groups with (20% Group A vs 24% Group B, $P=0.6$) and there was no significant difference in live birth and clinical pregnancy rates per initiated cycle between the two groups, 6.4% Group A vs 10.6% in Group B and 12% (A) vs 17% (B) respectively. Cycle cancellation rates were also similar between the two groups, 36% (A) vs 31% (B).

Conclusion: The trial shows that a prolonged 8-day course of CC does not suppress premature LH surge in an ART programme.

Keywords: Clomiphene Citrate, premature LH surge, IVF, ART, pregnancy outcomes

BACKGROUND

Oral CC is the first choice treatment for unexplained infertility and ovarian dysfunction with anovulatory cycles [1,2]. The choice is simply motivated by its effectiveness in inducing ovulation (ovulation rates of 70 – 80%) [3]. Added advantages include oral administration, known safety profile and being inexpensive [1]. However CC is reported to be associated with detrimental anti-oestrogenic effects on the endometrium and the cervical mucus [4]. Added to this negative concern is the increased risk of premature LH surge associated with CC usage. The incidence of premature LH surge is reported to be as high as 30% (15-30%) [5-8]. This may lead to impaired oocyte quality, lower pregnancy rates and increased miscarriages rates [9-12].

Trounson *et al.* reported a live birth rate per transfer of 8/23 (17%) with the use of CC alone in IVF [13]. Subsequently other authors have also reported positive outcomes of CC alone in IVF such as a high chance of oocyte retrieval (81%) [14] and a clinical pregnancy rate of 32.8% (21/64) per retrieval [15].

With the widespread increase in the usage of gonadotropins in the late 1980s and early 1990s, CC was used as an adjunct together with gonadotropins, with intent to increase the number of oocytes at the time of retrieval but keeping the number of gonadotropins low, thereby reducing the cost of treatment. The sequential or combined use of CC and gonadotropin protocols was reported to be associated with good clinical pregnancy rates, as high as 40% [16-18]. But the reported high cancellation rates of approximately 17% remain a concern [16]. In addition, the fewer oocytes retrieved per cycle and fewer embryos available for transfer as a result of these regimes have motivated the introduction of hypothalamo-pituitary suppression protocols with GnRHa, which eliminated the premature LH surge and led to multi-follicular recruitment and development [19]. However, this agonist protocol requires excessive amounts of gonadotropins, the treatment duration is long and exhausting and it results oestrogenic deprivation (hot flushes, vaginal dryness, headache) with overall stress for individual patients [5]. As an alternative to mitigate the above-mentioned effects of long GnRHa protocols, the mid-cycle GnRH antagonists were introduced in IVF, providing immediate suppression of pituitary gonadotropin for a

certain period of time [20]. It is important to note that the use of GnRH antagonists when compared with GnRHa in terms of probability of LBR and OPR, there were no significant differences between the two protocols [21,22].

In limited resource settings the described pituitary suppression protocols remain expensive and therefore make treatment unaffordable, further encouraging clinicians to explore other strategies to prevent premature LH surge and high cancellation rates in CC and gonadotropin ART cycles. Previously described methods to reduce the risk of premature LH surge includes prolonged use of CC until the day of hCG trigger [23], simultaneous administration of gonadotropins and oral progesterone beginning on menstrual cycle day 3 during ovarian hyperstimulation for IVF [24-26], pre-treatment with OCP prior to stimulation with CC [15] and/or simultaneous use of CC and a gonadotropin protocol [7]. The limitation of these strategies is that they lack robustness with evidence gathered from retrospective data or studies.

We therefore conducted a prospective RCT, comparing traditional use of CC of 5 days starting from cycle day 3 to 7 with prolonged use of CC for 8 days starting from cycle day 3 to 10 in a sequential gonadotropin cycle without mid-cycle GnRH antagonist.

Materials and Methods

Study design and setting

This study is a prospective RCT performed at Tygerberg Academic Hospital, Reproductive Medicine Unit, between June 2011 and December 2014, involving 349 patients who were candidates for ART in the form of IVF or ICSI. The study was approved by the ethics committee of Stellenbosch University, Faculty of Medicine and Health Sciences with reference no N11/08/256. We included female patients aged between 18 and 40 years of age. It had to be their first IVF treatment with regular menstrual cycles and a BMI (kg/m²) of 18-30. All causes of infertility were included. Patients with previously failed IVF treatment, presence of large ovarian cyst ≥ 3 cm on ultrasound, congenital uterine anomalies, severe endometriosis, BMI >35 and ovulatory dysfunction were excluded from the study. All participating patients signed a written consent form.

Treatment protocols

The patients were randomised into one of two treatment groups using a simple randomization schedule assigned via numbered sealed envelopes following patient assessment.

In group A, the patients were stimulated with CC (Fertomid, Cipla MEDPRO RSA) 100mg for 5 days from cycle day 3 to 7. hMG (Menopur, FERRING, SA) 150-225IU was added on cycle day 4, 6, 8 and/or 10.

In group B patients were stimulated with CC (Fertomid, Cipla MEDPRO RSA) 100mg for 8 days from cycle day 3 to 10. hMG (Menopur, FERRING SA) was added on cycle day 4, 6, 8 and/or 10. No baseline endocrine biochemical testing was performed in either group. Ultrasound was performed in both groups on cycle day 3 for AFC and evaluation for any cyst formation. The subsequent ultrasound was performed on cycle day 8 or 9 and thereafter according to follicular growth. LH was assessed from cycle day 9 and if negative, performed on alternative days until the day of hCG administration (Ovitrelle 250µg/0.5ml, Merck SA). If LH positive, the oocyte retrieval would be performed within the next 24 hours. hCG (Ovitrelle, Merck SA) 250µg/0.5ml was administered when there was a leading follicle of ≥ 18 mm.

Endometrial thickness was measured on the days of follicular assessment and the day of hCG administration. No serum oestradiol (E₂) levels were performed in either group. Oocyte retrieval was performed 34-36 hours after hCG administration by ultrasound-guided puncture of follicles, and IVF or ICSI was performed. All the embryos were assessed and scored morphologically before transfer [27].

ET was performed on day 2, 3 or 5 under ultrasound guidance with a Sage ET catheter. Luteal support with vaginal progesterone (Utrogestan, Medi Challenge SA) 400mg daily was started on the day of embryo transfer until the day of quantitative pregnancy test. Cycles were cancelled if no follicular growth occurred, no dominant follicles were present and with poor embryo growth.

Implantation was confirmed by measuring serum β -hCG levels on days 10 and 12 after ET. CPR is defined as the ultrasound presence of foetal cardiac activity at 7 weeks of gestation and OPR as the presence of foetal cardiac activity at ≥ 12 weeks of gestation. LBR is also defined as the birth of a singleton, live baby at term.

Primary outcome measure was the rate of premature LH surge between two groups, and secondary outcome measures were CPR, LBR, cancellation rates and miscarriage rates.

Data and statistical analysis

Results are presented as the median and the range unless otherwise indicated. Comparison of outcome measures was performed using a *t* test for symmetrical continuous data and the *Mann-Whitney test* for non-symmetrical continuous data. The χ^2 test was used for binary variables. The trial comparing two treatment regimens was designed to detect a difference of at least 20% (power 80%) in LH surge between the 5 days and 8 days of CC administration. Two hundred and twenty eight patients should be included to achieve this aim. P-value < 0.05 was considered the limit of statistical significance. Data was analysed using STATA 13 Software.

Results

There were no significant differences between the groups with regard to demographic characteristics such as age and indication for treatment (Table 1). Two hundred and thirty eight (238) patients were enrolled and randomized. In the 5-day protocol of CC, 5 patients were excluded and we lost follow-up in 1 patient. In the 8-day protocol of CC, 5 patients were excluded but we did NOT lose any patients to follow-up. Therefore we analysed 124 patients in the 5-day CC group vs 103 patients in the 8-day CC group (Figure 1).

There was no significant difference in the number of oocytes and number of metaphase II in the two groups. [2(0-12) vs 3(0-15), P=0.16] and [2(0-11) vs 3(0-14), P=0.15] (Table 2).

However, there was a significantly higher fertilization rate in the long 8-day protocol vs the short 5-day protocol [1(0-10) vs 2(0-13), $P = 0.04$]. The number of good quality embryos was similar in both groups (1.5 ± 1.0 vs 1.8 ± 0.9 ; CI 95% 1.51–1.8). The percentage risk of premature LH surge was also similar in both groups. (20% vs 24%, $P=0.6$) and there was no significant difference in clinical pregnancy rates between the two groups. (12% vs 16%, $P=0.3$) No cases of OHSS were reported during the study period.

Conventional IVF and ICSI were used in the same percentage of cycles in both groups (Table 2).

There were similar high cycle cancellation rates in Group A and Group B, due mainly to poor embryo development and no embryos available for transfer. There was no difference in the endometrial thickness between the two groups and the number of gonadotropins used in both groups was also similar.

Figure 1: Recruitment, randomisation, follow-up and drop-outs over the period of the study

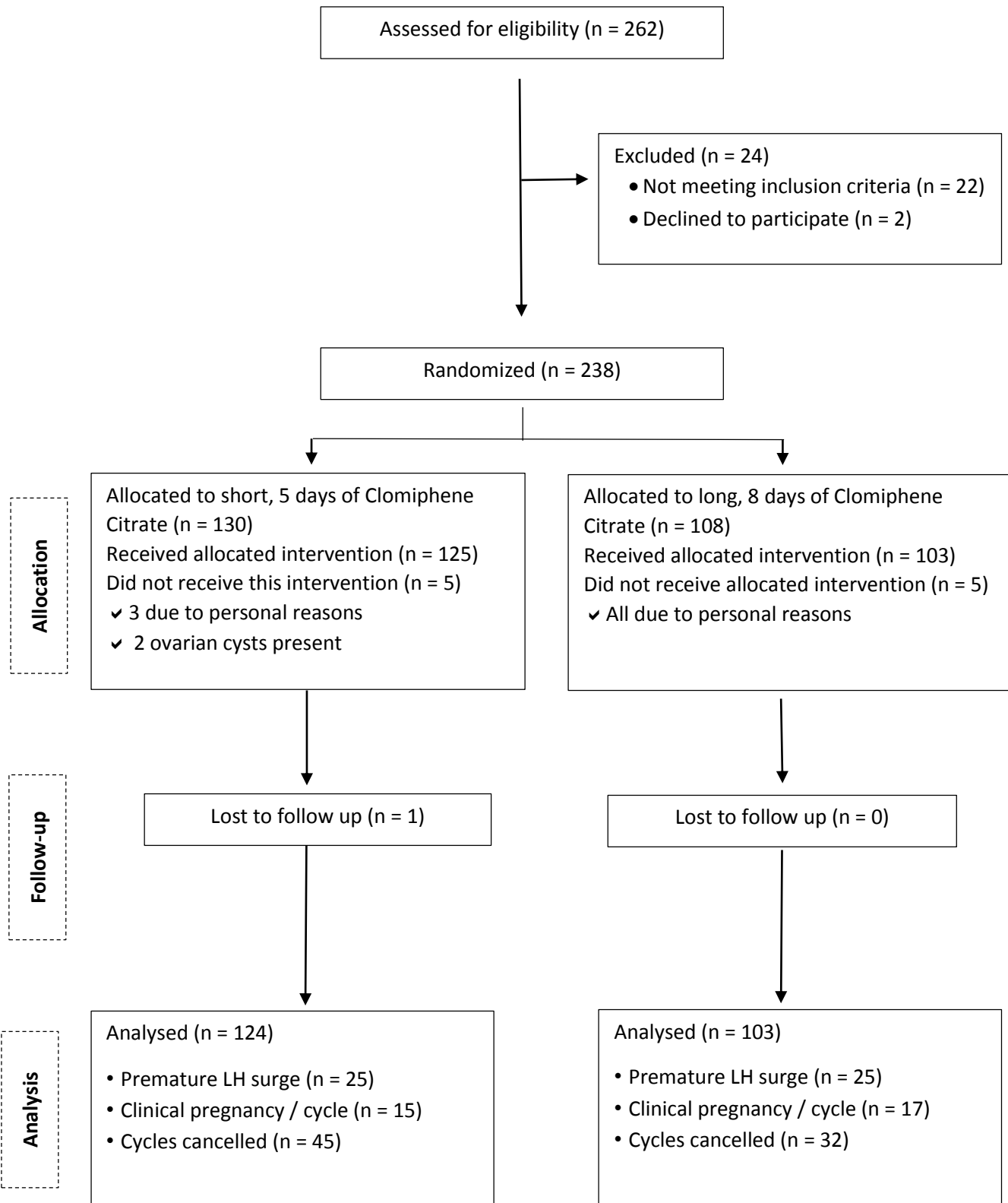


Table 1: Demographic characteristics of patients undergoing an IVF/ICSI treatment in Clomiphene Citrate protocol, randomised for two different regimes.

Variables	5 days group A (n = 124)	8 days Group B (n = 103)	<i>P-value</i>
Age (years) ^a	35.5 ± 4.9	35.3 ± 4.3	0.37
BMI (kg/m ²) ^a	28.3 ± 2.6	28.1 ± 2.1	0.26
Infertility causes			
• Tubal factor	56 (45%)	42 (40.7%)	0.2
• Teratozoospermia	31 (25%)	26 (25.2%)	0.5

BMI = Body mass index, ^aMean ± SD

Statistically significant values (P < 0.05)

No significant difference between two groups.

Table 2: Clinical outcomes of the two different treatment groups

Variables	5 Days, Group A (n = 124)	8 Days, Group B (n = 103)	Between group differences (Confidence limit)	P Value
Number of oocytes ^a retrieved	2 (0 – 12)	3 (0 – 15)		0.16
Number of MII oocytes ^a	2 (0 – 11)	3 (0 – 14)		0.15
Number of oocytes fertilized (Fertilization rate) ^a	1 (0 – 10)	2 (0 – 13)		0.04 ^b
Number of embryos transferred ^a	1 (0 – 4)	2 (0 – 4)		0.06
Endometrial thickness (mm) ^c	8.6 ± 1.3	8.5 ± 1.2	(CI 95%: -0.2 – 0.4)	0.27
Day of embryo transfer ^c	2.9 ± 0.9	3.0 ± 0.8	(CI 95% : 2.8 – 3.1)	0.8
Good quality embryos available for transfer ^c	1.5 ± 1.0	1.8 ± 0.9	(CI 95% 1.51 – 1.8)	0.9
Premature LH Surge (Positive urinary LH before HCG administration)	CI 95% (1.2 – 1.7) 25 (20%)	CI 95% (1.6 – 2.10) 25 (24%)		0.6
Clinical pregnancy rate per started cycle	15 (12%)	17 (16.5%)		0.3
Clinical pregnancy rate per embryo transfer	15 (19.4%)	17 (24%)		0.5
Live birth rate per cycle	8 (6.4%)	11 (10.6%)		0.2
Live birth rate per embryo transfer	8 (7.8%)	11 (15.5%)		0.3
Miscarriage rate	7 (46.6%)	6 (35%)		0.9
Cycle cancellation rate	45 (36%)	32 (31%)		0.4
OHSS	0 (0%)	0 (0%)		
ICSI	51 (41%)	44 (42.7%)		0.68
IVF	73 (58.8%)	56 (54%)		0.66

MI I = Metaphase II, LH = Luteinizing hormone, HCG = human chorionic gonadotropin, OHSS ovarian hyperstimulation syndrome
a = median values reported, b = statistically significant value (pvalue =0.04), c = data presented as the mean ± SD, mm = millimeter

DISCUSSION

The introduction of GnRH agonist and GnRH antagonist in IVF/ICSI cycles was to eliminate the risk of premature LH surge which was reported to be approximately 20-25% in CC and/or gonadotropin cycles [6, 28]. However these protocols require significantly large amount of gonadotropins at a very high cost [29]. In the present randomised trial, we recorded an early LH surge (positive urinary LH prior hCG administration) as high as 20% in the five-day course of CC (group A) vs 24% in the eight-day course of CC (group B). The findings are similar to those reported in other studies [5, 28]. The fundamental issues of concern with premature LH surge include high cycle cancellation rates, unplanned oocyte retrieval, poor timing for oocyte maturation, resulting in aged oocytes and a detrimental effect on the endometrium preparation for implantation with overall lower pregnancy rates [4,5,12].

Amongst the less expensive proposed methods to prevent premature LH surge [7,15,23-26]. Kawachiya *et al.* in a retrospective study of 543 cycles, using 50mg CC from cycle day 3 and 75IU hMG every other day from cycle day 8 until the leading follicle developed to 18mm in diameter and administration of CC stopped at the time of oocyte maturation trigger with 300 microgram (μ g) GnRH agonist (Buserelin), reported low rates of premature LH levels (5%) [23]. The conclusion of the study was that prolonged usage of CC is sufficiently effective to suppress the premature LH surge in minimal stimulation IVF protocol. On the contrary, in the current trial the rate of premature LH surge in a prolonged CC arm was as high as 24%.

In the current trial, LH testing was performed once daily from cycle day 9 up to the day of hCG administration using the collected urine sample. The aim was to reduce the costs related to serum blood testing. Several studies have reported high sensitivity rates ranging from 85% to 100% of urine LH tests to predict ovulation in advance [30]. It has since been recognized as an acceptable method used by patients to assess LH peaks in order to time intercourse and also by physicians during trials and/or treatment cycles for infertility [31-34].

The high cycle cancellation rates of 36% and 31% in Groups A and B of this study respectively were largely due to cancelled embryo transfers as a result of poor embryo development and growth. Cycle cancellation for poor follicular growth was 10% (Group A) and 11% (Group B). The selective and strict transfer of good quality embryos could explain the overall high cycle cancellation rates and low miscarriage rates (Table 2) in this study. The miscarriage rate in CC treatment is reported to range from 13 to 25% [12]. All these negative outcomes are attributed to premature LH surge. In both Group A and B, the oocyte retrieval was achieved in 90% and 89% respectively, with no significant difference in the number of oocytes retrieved ($P=0.16$) and the available number of metaphase II oocytes ($P=0.15$). Therefore we did not cancel the cycle simply because of an early LH surge, and acceptable rates of oocyte retrieval were observed. This practical approach has also been described in the literature [35].

Although there was a significantly higher number of fertilized oocytes in Group B [1 (0-10) vs 2(0-13), $P=0.04$], the available number of good quality embryos for transfer was not significantly different in both Group A and B. [1.5 ± 1.0 vs 1.8 ± 0.9 , CI 95% 1.51 – 1.8). The clinical pregnancy rates per started cycle of (12% vs 16.5%, $P = 0.3$) and the LBR per transfer of (7.8% vs 15.5%, $P=0.3$) were lower when compared to pituitary suppressed protocols results. Similar pregnancy outcomes have previously been reported in CC treatment cycles [7,13]. These low pregnancy outcomes could be explained by high levels of early LH surge in this study. Furthermore, it is important to note that despite a high oocyte retrieval rate and satisfactory fertilization rate, the high cancellation rate due to poor embryo development might be a reflection of the impact of high LH on the oocyte quality [5]. There was no difference in the endometrial thickness in both groups, with the average thickness of 8.5 ± 1.2 mm at the time of hCG administration. Previous studies have also reported no detrimental effect on the endometrial growth and morphology in CC and/or gonadotropins ART treatments [36,37]. And no cases of OHSS were reported in this study.

Financial and geographic limitations are profound barriers to the use of ART and favour CC treatment amongst low socio-economic status and rural women [2]. This phenomenon is shown in the increased usage of CC in women between 30-44 years of age, indicating a growing demand for fertility treatments in this population [2]. The use of aromatase inhibitors such as Letrozole as a substitute or an alternative to CC

is still debatable because of similar ovulation rates with no significant benefit observed other than associated high costs of the drug [38]. The fact remains, in low resource settings, GnRH agonist and/or GnRH antagonist can never be easily affordable. Therefore the use of CC in conjunction with gonadotropins will remain an alternative, if not the first line of treatment. The ongoing research and effort should strive towards achieving acceptable pregnancy rates in these protocols as shown by Williams *et al.* and Weigert *et al.* [16,39]. Furthermore, affordable strategies to lower the risk of premature LH surge should continue to be evaluated in robust clinical trials. To our knowledge, this study is the first randomised controlled trial to evaluate the role of prolonged CC usage as a strategy to prevent premature LH surge in IVF/ICSI cycles.

In conclusion, this trial shows that prolonged usage of CC from cycle day 3 to cycle day 10 did not suppress the premature LH surge in a mild ovarian stimulation protocol using CC and gonadotropins IVF/ICSI treatment. Therefore other methods such as oral contraceptive pre-treatment and simultaneous progesterone administration should also be evaluated in larger and robust trials.

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CHAPTER 4: CHILDLESSNESS IN A LIMITED-RESOURCE COUNTRY; A PAINFUL SUFFERING TO ENDURE: PUBLIC-PRIVATE INTERACTION, A MODEL TOWARDS MAKING ASSISTED REPRODUCTION ACCESSIBLE

SYNOPSIS

Objective: To evaluate PPI as a strategy to make ART affordable

Design: Prospective descriptive study

Setting: Tygerberg Academic Hospital, Reproductive Medicine Unit

Population: All women ages 20-45 years attending the fertility clinic for ART (IVF/ICSI) treatment for female and male infertility problems

Methods: Clinical treatment records of all eligible participants were analysed. Treatment involved CC 100mg from cycle day 3 to 7 or day 3 to 10 in combination with alternate day administration of hMG 150-225IU from cycle day 4, 6 and 8. hCG was administered when the leading follicle of ≥ 18 mm was present and the retrieval followed 34-36hrs later. Cycle monitoring included the ultrasound and urinary LH testing with no use of any biochemical endocrine blood tests.

Main outcome measures: LBR, CPR and also the direct costs of treatment to individual couple per cycle of ART.

Results: Three hundred and seventy five (375) cycles of ART were performed and 346 (92%) reached oocyte retrieval stage with a fertilization rate of 72.5%. The LBR per ET and per cycle started were 17.7% and 10.6% respectively, while the CPR per ET and per started cycle were 24% and 14% respectively. A cycle cancellation rate of 40% was observed. A multiple pregnancy rate of 5.6% was recorded and there were no cases of OHSS. Direct costs of treatment per cycle were R7291 (563USD) (cycle costs R6000 – R8000). A cumulative pregnancy rate of 32% following three cycles of ART was also observed.

Conclusion: The study shows that PPI can be a possible and viable strategy to make ART affordable and accessible with reasonable pregnancy rates at low cost. The model can be implemented, reproduced and be sustained.

Keywords: IVF, ICSI, ART, developing countries, limited resources, public private interventions

INTRODUCTION

Infertility is undeniably a public and global health issue [1]. With over 5 million children being born following ART worldwide, this technique is still not widely available or used in either developing and developed countries because of its high costs [2]. The availability of funding or health insurance facilities for ART differs worldwide with optimal coverage in Belgium to absolutely no cover in the United States of America (USA) [3-5]. Cost is the major limiting factor and number one deterrent for infertile couples to seek ART treatment [6,7]. The cost drivers of IVF cycle in a relatively large private clinic are mainly medication (28%), clinicians' fees and consultation (29%), and laboratory fees (35%) [8]. The first report on ART data monitoring in South Africa and sub-Saharan Africa (developing countries) shows that the ART needs of many couples are still unmet, with only 6% coverage [9]. This issue receives very little attention because most governments and authorities in the developing countries are faced with major health challenges such as high maternal morbidity and mortality, and infectious diseases including TB, HIV and malaria [10].

However the desire to have children especially in the developing countries presents much stronger negative psychosocial consequences from psychological distress, domestic violence, stigmatization and polygamy [11-13]. And couples will go to great length and difficulty to achieve this goal. Studies have shown that to have a healthy child, couples may accept a 20% risk of death and give up 29% of their income [14]. Chambers *et al.* further reported that one fresh cycle of IVF accounts for 52% of an individual's average disposable income in states without ART insurance mandate [6]. There are more than 80 million couples affected by infertility worldwide and the majority of this population resides in the developing countries where funding for ART does not exist [15]. This protracted and painful undesired situation of childlessness for millions of couples in the developing countries has been well expressed by Murage *et al.* in a cross-sectional survey, showing that 26.1% of gynaecologic consultations in Kenya were related to subfertility and 50.3% were due to tubal factors, while 14.8% were due to male factors [16]. Simply, implying that more than 50% of fertility related problems were likely to require ART that is severely limited to only three private units at an exorbitant cost [16], thus expressing the dire demand for ART services in

developing countries. It has been shown that by reducing the cost of treatment and improving access to treatment is associated with improved patient safety and reduction in undesirable complications of high order multiple pregnancies [3, 17]. This situation is to be avoided at all possible costs in poorly resourced countries.

The current paper seeks to evaluate and demonstrate PPI as a possible strategy to make ART accessible in the very limited resources settings by describing a series of patients managed through the model.

PART A: The description of Public Private Interaction Model

Public-private interaction is a model that involved negotiations and a signed level of agreement (SLA) between public sector hospital, Tygerberg Academic, and private clinic Drs Aevitas Institute of Reproductive Medicine, in Cape Town (Addendum A).

Personnel

Tygerberg Academic Hospital unit initially began functioning only with the fertility specialist in charge of the programme (Dr Thabo Matsaseng) and two embryologists (Dr M Windt-de Beer and Mrs E Erasmus), who are full-time employees of Stellenbosch University and the Provincial Government of the Western Cape, respectively. The cycle bookings and arrangements were handled by the doctor in charge of the treatment, thereby eliminating clinic and administrative fees. The unit currently has an additional fertility specialist and a junior embryologist who were not part of the study. The newly recruited embryologist is the person now in charge of cycle's co-ordination and bookings.

Ovulation induction: Mild Ovarian Stimulation protocol (Figure 1)

The patients were stimulated with CC (Fertomid, Cipla MEDPRO RSA) 100mg for 5 days from menstrual cycle 3 to 7 and hMG (Menopur, FERRING, SA) 150-225 IU (2-3 ampoules) that was added on cycle day 4, 6, 8 and/or 10. We generally do not use more than eight ampoules in total (1 ampoule =75IU). Ultrasound was performed on day 1-3 of the menstrual cycle before treatment was initiated. This was to evaluate the

AFC and to rule out ovarian cysts. Follicular monitoring began on cycle day 8 and was performed every alternate day until the day of hCG administration. Urine LH tests were performed from cycle day 9 and every alternate day and sometimes daily until the day of hCG administration. No endocrine biochemical tests were prepared as part of monitoring. In cases where spontaneous ovulation is highly suspected based on follicular size (>22mm) and weak positive urinary LH before hCG administration, NSAIDs Indomethacin (Arthrexin, Adcock Ingram SA) 25mg three times a day will be offered until the day of oocyte retrieval [18]. hCG (Ovitrelle, Merck SA) 250mcg/0.5ml was administered where there was a leading follicle of ≥ 18 mm. Oocyte retrieval was performed 34-36 hours after hCG administration by ultrasound guided puncture of follicles, and IVF or ICSI was performed.

Laboratory – oocyte retrieval, insemination and embryo transfer

Oocyte retrieval is done under conscious sedation without the need for anaesthesia, an anaesthetist or a theatre set-up. Patients receive Pethidine 100 mg intramuscularly 15-30 minutes before the procedure and a 20ml local block with 1% Lignocaine without Adrenaline. They recover in a room near the laboratory for approximately 30 minutes after the procedure and are discharged in the company of a family member once they are fully awake. Sperm will be prepared on the day of oocyte retrieval using 3-layer density gradient centrifugation (90%–70%–40%) (PureSperm, Nidacon) followed by two washing steps in Earle's Balanced Salt Solution + 5% Human Serum Albumin (HSA). Samples will be re-suspended in Global[®] (G-IVF, Life Global, USA) + 5% HSA and incubated at 36.5°C until fertilization. Oocyte-cumulus-complexes were placed in a 5ml tube with 1ml Global[®] for fertilization + 5% HSA (pre-equilibrated) and a volume equivalent to 50 000 to 100 000 motile sperm added. Fertilization and embryo culture will occur in a tissue culture incubator in an atmosphere of 5% CO₂ in air, with confirmation of fertilization made 16-20 hours later. All embryos were assessed and scored morphologically before transfer [19]. ET was performed on day 2 or 3 under ultrasound guidance with a Sage ET catheter. Occasionally ET was performed on day 5. Cryopreservation facilities are available when necessary. Consumables such as aspiration needles and embryo transfer catheters were provided by the private clinic following single use and therefore were properly sterilized before being re-used. This

was to further lower the cost of treatment without compromising patient safety and results.

In summary, we were convinced that the model will assist in cutting costs at the level of medication, clinicians' fees and laboratory fees, which are the biggest cost drivers in IVF treatment. The model was published in the local journal, SAJOG 2014; 20: 33-34. Doi: 10.7196/SAJOG.814 and non-peer reviewed journal, Specialist Forum 2014. It also attracted a wide media interest with television broadcast on the BBC news channel (internationally) and eNCA news bulletin (national broadcaster). This has led to a request for the model to be evaluated for implementation by a local tertiary institution, Chris Hani Baragwanath hospital in Johannesburg via Dr Tshabalala. Through this model we describe the outcomes of patients managed by ART at Tygerberg Academic Hospital.

PART B: A description of the first 375 cycles managed with ART through PPI model.

Materials and Methods

Design: Pragmatic, prospective descriptive study

Setting: The ART treatment was provided by the Reproductive Medicine Unit at Tygerberg Academic Hospital (public)

Data collection

The study included all women who underwent ART in the form of IVF and ICSI in our unit from 2011 to 2014. It was couples who required ART irrespective of the diagnosis, age (limit ≤ 42 yrs of age), BMI (kg/m^2) [limit ≤ 36]. There was no specific exclusion criterion for couples in their first cycle of ART. The patients were counselled on the treatment protocol, the cost of treatment and the success rates that are lower than those of conventional ART programmes.

Mild Ovarian Stimulation protocol (Figure 1)

The patients were stimulated with CC (Fertomid, Cipla MEDPRO RSA) 100mg for 5 days from menstrual cycle 3 to 7 and hMG (Menopur, FERRING, SA) 150-225 IU (2-3 ampoules) that was added on cycle day 4, 6, 8 and/or 10. We generally do not use more than eight ampoules in total (1 ampoule =75IU). Ultrasound was performed on day 1-3 of the menstrual cycle before treatment was initiated. This was to evaluate the AFC and to rule out ovarian cysts. Follicular monitoring began on cycle day 8 and was performed every alternate day until the day of hCG administration. Urine LH tests were performed from cycle day 9 and every alternate day and sometimes daily until the day of hCG administration. No endocrine biochemical tests were prepared as part of monitoring. In cases where spontaneous ovulation is highly suspected based on follicular size (>22mm) and weak positive urinary LH before hCG administration, NSAIDs Indomethacin (Arthrexin, Adcock Ingram SA) 25mg three times a day will be offered until the day of oocyte retrieval [18]. hCG (Ovitrelle, Merck SA) 250mcg/0.5ml was administered where there was a leading follicle of ≥ 18 mm. Oocyte retrieval was performed 34-36 hours after hCG administration by ultrasound guided puncture of follicles, and IVF or ICSI was performed.

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confirmation of fertilization made 16-20 hours later. All embryos were assessed and scored morphologically before transfer [19]. ET was performed on day 2 or 3 under ultrasound guidance with a Sage ET catheter. Occasionally ET was performed on day 5. Cryopreservation facilities are available when necessary. Consumables such as aspiration needles and embryo transfer catheters were provided by the private clinic following single use and therefore properly sterilized before being re-used. This was to further lower the cost of treatment without compromising patient safety and results.

Luteal support with vaginal progesterone (Utrogestan, Medi Challenge SA) 400mg daily was started on the day of ET and continued until the day of the quantitative pregnancy test. The cycle was cancelled if there was no follicular growth, no oocytes retrieved during aspiration, no fertilization and poor embryo growth. Implantation was confirmed by measuring serum β -hCG levels on day 10 and 12 following ET (≥ 25 IU on day 10 confirmed pregnancy).

CPR is defined as the ultrasound presence of foetal cardiac activity at 7 weeks of gestation and OPR as the presence of foetal cardiac activity at ≥ 12 weeks of gestation. LBR is defined as the birth of a singleton, live baby at term.

The primary outcome measure was to show whether public-private interaction can make ART affordable, looking at the cost of treatment cycle and the outcomes, in terms of live birth rate.

Secondary outcomes include the number of oocytes retrieved, fertilization rates, clinical pregnancy rates, miscarriage rates and ectopic rates.

The study was approved by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences (FMHS) of Stellenbosch University with reference no N11/08/256.

Data and Statistical analysis

Data are presented as percentages (n) and means \pm SD or range.

The Kaplan-Meier survival estimates were used for prediction of pregnancy probability given the number of treatment cycles.

The student t-test was used to compare the means and a p-value of <0.05 was considered to be statistically significant. All analyses were performed with Stata 13 software.

Results

A total of 375 cycles were performed with this low cost model between 2011 and 2014. The average age of women was 34.9 ± 4.7 (oldest was 44 yrs) and the most common indications for treatment were tubal disorder (45.6%) and teratozoospermia (28.6%) [Table1]. A total of 346 cycles (92%) reached oocyte retrieval stage. The cycle cancellation rate in this study was 8%. At the time of aspiration no oocytes were obtained in 10.7% (37/346) of the cases despite the presence of the expected number of follicles, and in 16.7% of the cases there was no fertilization. In addition, 7.5% of the cases did not reach embryo transfer stage as a result of poor quality embryos (Table 2). We have observed that cycle cancellations as a result of poor follicular development and absence of oocytes at the time of aspiration (in the presence of follicles) occurred more frequently in patients with PCOS, previous cystectomy for endometrioma and advanced female age of >38 yrs old.

IVF and ICSI were performed in 202 (53.8%) and 167 (44.5%) cycles respectively. The mean number of oocytes retrieved was 4.3 ± 4.0 (0-20). There was fertilization failure in 58 (16.7%) cycles. There were significantly more failures with IVF, 38/58 (65.5%) than ICSI, 20/58 (34.5%) $p=0.0008$. The mean number of embryos transferred was 2.99 ± 0.93 (range 1-4), with 77% (389/503) of good quality embryos available for transfer. A clinical pregnancy rate of 14.1% (53/375) per started cycle and 23.5% (53/225) per ET was achieved. Twelve clinical pregnancies ended in miscarriages 22.6% (12/53) and 1 ended as an ectopic pregnancy (tubal) (1.9%). Therefore a total

of 40 babies were born, giving a LBR per stated cycle of 40/375 (10.6%) and LBR per ET of 40/225 (17.7%).

A multiple pregnancy rate of 5.6% (3/53) was recorded and it was only twins, with no triplets observed. No cases of OHSS were recorded (Table 3).

The probability of achieving a pregnancy following one, two and three cycles of IVF were 14.1%, 19.8% and 31.7% respectively (Table 4, Figure 2). There was no improvement in pregnancy chance between cycles 4 to 6 of treatment.

The overall cost of IVF/ICSI cycle is R7 291.00 (563USD) which is direct cost to patient, and in the private clinics the cycle cost varies between R35 000 and R50 000 (3 861 USD) (Table 5).

Further cost comparison shows that infrastructure (rental fees), personnel salaries, medication and laboratory costs are the greatest cost drivers in IVF/ICSI treatments (Table 5).

DISCUSSION

There is an increasing demand, utilization and applications of ART worldwide owing to both the increasing effectiveness of treatment and the increasing rates of subfertility as couples delay childbirth [6]. The rising trend of utilization has been observed in the developed countries, particularly where there is public funding for ART [20]. However in the developing countries access to ART remains nothing but a dream to be realised [21], largely because of the high cost and unaffordability of treatment [6,7,16]. The strategies developed in an attempt to make ART affordable are not intended to compromise on quality, but at least to improve access to many who would otherwise be denied the opportunity and joy of parenthood [21, 22].

In this current study we present a PPI model in a limited resource setting. According to the draft health charter, PPI is defined as one or more persons or entities involved in health care within the public sector interacting with one or more persons or entities involved in health care within the private sector or the non-governmental organization

sector with the intention to achieve mutual benefit. This includes public-private partnership (PPP) and PPI [23]. A PPP involves a contractual agreement between public and private parties in which the private party performs an institutional function or uses state assets and assumes substantial financial, technical and operational risk of the project in return for a benefit [23], and it requires participation, registration and approval of the National Treasury. However, any project that does not require any involvement of the National Treasury should be regarded as PPI [25], as illustrated in our model which is a contractual SLA signed between the hospital and the private sector. The relationship may be a once-off involvement or be on an ongoing basis.

An optimally functioning laboratory is a prerequisite to ensure good results and the public hospital (Tygerberg Hospital) provided the infrastructure in which the clinic and the laboratory provided the platform for consultation, monitoring and performance of ART treatment. The hospital billed the patients according to their level of salary income for every visit to the clinic (Addendum A). In the private setting this infrastructure is often a property that needs to be rented out at a cost that will be borne by individual patient (Table 5). Furthermore, the FMHS of Stellenbosch University contributed towards the laboratory infrastructure development through the purchase of the incubator, and the private clinic (Drs Aevitas) also donated the old ICSI machine and incubator to the laboratory. These are significant contributions that have to be acknowledged, especially when the actual cost of this equipment is known to be in the millions [8]. As previously mentioned, the interaction may not necessarily be ongoing, but a once off contribution that is significant and meaningful is also recognized. In this regard the contribution by Tygerberg Hospital (providing infrastructure at a low cost to the patients), the Stellenbosch University FMHS and Drs Aevitas (providing necessary and expensive equipment at no cost to patients) is a point in illustration on how PPI contributes to lowering the cost of ART in the long term as there are no loans to be repaid. These interactions have been observed in other countries like Brazil [26]. Although ICSI treatment might be required for severe male infertility, during the initial set-up IVF is sufficient to provide the necessary service, especially as the majority of infertility is caused by tubal factors (45.6%).

Personnel or staff competence and commitment is vital to successful implementation of effective ART services [27]. In this current model the certified reproductive medicine

specialists and embryologists are the employees of the University and the Government respectively. Therefore their remuneration is fully subsidised by their respective employers at no direct cost to patients (Table 5). In the private setting the clinician's fees and consultation contribute 29% (R14 500 / 1 120 USD) towards IVF cost, and this is a direct cost to the individual couple. There are no IVF sisters, nurses or co-ordinators in this currently presented model, which required an additional effort from the available staff to co-ordinate and provide the service. In this regard, it is Tygerberg Hospital and Stellenbosch University FMHS (governmental, public institutions) contributing towards lowering the direct costs of IVF to individual couples. At the beginning, the service can adequately be performed by one doctor and one embryologist.

In this study, we used a combination of CC and hMG for ovarian stimulation at a cost of R3 500–R4 000 (270-308 USD) (Figure 1) as opposed to conventional ovarian stimulation at a cost of R14 000 (1 081 USD) [8]. Mild ovarian stimulation protocols are defined in the literature [28,29] and their associated advantages and disadvantages are also well expressed [30,31]. We did not use the GnRH antagonist in this protocol as a measure to lower the cost of treatment. Similar protocols such as ours have been used with reasonable success [32]. The cycles in this current model were monitored with an ultrasound and urinary LH test only. Due to lack of clear evidence to suggest any significant benefit in improving IVF outcome or predicting OHSS probability, the biochemical endocrine blood tests were not performed [33,34]. Palmer *et al.* have shown with a high degree of sensitivity that urine LH tests can predict ovulation in advance [35]. The cost of oocyte retrieval was also lowered quite significantly, by performing the procedure in the ART laboratory with adequate monitoring and resuscitation facilities nearby. The patients were offered 100mg of pethidine injection 15-30 minutes before procedure and were also injected locally with 1% Lignocaine at the puncture site. This approach eliminated the need for a theatre and anaesthetist (Table 5).

Laboratory costs account for 35% (R17 500 / 1 351 USD) of the IVF cycle in private clinics [8]. In our model the couple pay for consumables such as media, plastics and slides on average R364 (28 USD) per cycle of IVF/ICSI. There are no costs related to theatre set-up and an anaesthetist for oocyte retrieval as described above. Again, the

doctors administering conscious sedation medication are the ones performing the oocyte retrieval, and the embryologist performs the role of the nurse as well. This is a significant way of further lowering the cost of IVF. This is a contribution by Tygerberg Hospital and Stellenbosch University FMHS (governmental, public institutions) by providing the necessary human resource and platform to perform ART procedures safely. The patients will also pay an additional fee of R500 (38.60 USD) for ICSI pipettes if necessary. More often, we use the sterilized aspiration needles and embryo transfer catheters to further lower the cost of treatment without compromising patient safety and results. The patients are always informed about the method and given an option to purchase new apparatus if so wish. These consumables are received from the private clinic following single use and they will undergo a rigorous cleaning and sterilization process with gamma radiation at a dose of 18 kilo Gray (kGy) [36-39]. Without this interaction with private clinic, the patients would be forced to buy new apparatus for every cycle of IVF/ICSI. This is another illustration on how PPI contributes towards lowering the cost of ART treatment. The total minimum cost of the IVF/ICSI cycle could amount to R122 326 (9 446 USD) in the public unit versus R187 000 (14 440 USD) in the private facility, and the couple's direct cost to treatment is approximately R7 291 (563 USD) versus R51 024 (3940 USD) for public and private facilities respectively (Table 6). These figures illustrate the significant contribution made by the government (hospital and/or university) through participation and commitment in the model together with the involvement of the private sector in lowering the cost of IVF treatment to individual state patients. It is important to note that the cost of ART services did not exceed 0.25% of total healthcare expenditure in any country providing this kind of service [4].

The breakdown of individual cost per cycle was:

- hospital visits (max 5) = R1 205 (93 USD)
- medication (stimulation and luteal support) = R3 500 (270 USD)
- laboratory fees = R1 427 (110 USD)
- plus R500 (39 USD) for pipettes.

During the study period 92% of started cycles reached an oocyte retrieval stage. We did not have a policy of cancelling the cycles based on the number of follicles, or

positive LH test before HCG administration. The mean number of oocytes retrieved per cycle was 4.3 ± 4.0 , which is lower than the number of oocytes collected in conventional IVF. Sunkara *et al.* reported that approximately 15 eggs are required in conventional IVF cycles to improve the live birth rate [40]. Despite the low number of oocytes retrieved, the mean number of good quality embryos available for transfer on day 2/3 in this study was $2.09 \pm 2.36 / 2.02 \pm 2.01$ respectively. Similar findings of a lower number of oocytes retrieved during mild ovarian stimulation cycle but which yielded a higher number of good quality embryos and satisfactory pregnancy results, were observed in a randomised controlled trial by Hohmann *et al.* [31]. Therefore other variables besides the number of oocytes associated with pregnancy outcomes in IVF should always be evaluated. The clinical pregnancy rate of 14.1% per started cycle and 23.5% per ET were achieved. These results are similar to those reported in the previous studies [22,41-43]. The live birth rates in this study were 10.6% per cycle and 17.7% per ET. Similar outcomes were observed in an affordable IVF study by Aleyamma *et al.* [22]. Williams *et al.* have reported ongoing pregnancy rates of 30% with a similar protocol, but no LBR were reported [32]. For women under the age of 35 years, in the public sector (low cost model) vs the ones in the private sector (conventional IVF, SARA report) [9], the clinical pregnancy rates per ET were significantly higher in the private sector group; 40% (private) [9] vs 25.4% (public), $p=0.0041$ (Table 3). Similarly in women >35 yrs of age the results were significantly better in favour of conventional IVF in the private sector, 32% (private) [9] vs 18.9% (public), $p=0.01$ (Table 3). This therefore suggests that if a couple can afford the private fees, the conventional IVF would be the recommended approach.

The proportion of absent oocytes at the time of aspiration was high in this study despite the presence of expected number of follicles. This finding does stimulate the debate around follicle flushing in patients with a lesser number of follicles [44]. Because of limited available evidence this is not a protocol in our unit and therefore a randomised controlled trial with the aim to evaluate the benefit of this strategy in the setting of a low number of oocytes is much needed. High fertilization failure rates (65.5%) were observed in IVF treatment cycles in this study despite the normal semen parameters. These findings may reflect the probability of poor oocyte quality. However there is also a possibility that ICSI would have resulted better fertilization rates, especially when dealing with a low number of oocytes. But this notion is not supported by the current

available evidence, that ICSI provides any additional benefit to couples with few oocytes in ART treatment [45,46]. Therefore severe male infertility should remain the primary indication for ICSI treatment. The cycle cancellations as a result of poor follicular development occurred more frequently in patients with PCOS, previous cystectomy for endometrioma and advanced female age of >38yrs old. It is therefore important to select the patients who would benefit from the mild ovarian stimulation regimen carefully and appropriately and further advise on the role of ovarian reserve testing and the need for timeous conventional ovarian stimulation IVF.

This low cost model study further shows that there were 237 first attempt cycles of ART and only 37 (9.8%) cycles were third time attempts, clearly demonstrating a significant drop in the number of treatment attempts. The financial limitation as a strong barrier towards treatment accessibility was the primary reason for the significant drop since the majority of the patients could not afford the second, let alone the third cycle of IVF (Table 2). In this study the couple had a 14.1% chance of pregnancy after the first cycle of ART, 19.8% after the second cycle and 31.7% after the third cycle (Figure 2). It means that for a couple to achieve results above 30% of pregnancy in a low cost model, they will need at least three cycles of ART, and this will cost them approximately R22 000 (1 698 USD). The probability of any significant improvement (above 32%) in pregnancy result may be realised after the sixth cycle of IVF (Figure 2). But it is also known that IVF does not guarantee success, with almost 50% of couples starting treatment who will remain childless, even if they undergo six cycles of IVF [19]. The available knowledge regarding ART treatment is that a plateau will be reached following 4 cycles, and the present standard of care is to consider other options beyond the 4th cycle. The index study shows a plateau (Table 4, Figure 2) following three cycles with a total of 22 attempts in cycles 4 to 6. Women in the public sector should be encouraged to budget for 3 cycles until more information is available. This study demonstrates that even though the pregnancy outcomes are lower in the low cost model than in conventional IVF, the model does provide access and hope to those couples who might not have had a chance at all to try and possibly succeed with IVF. And factors such as hydrosalpinx and increased body weight should be dealt with prior to initiation of ART treatment as they were some of the factors thought to be negatively affecting the pregnancy outcomes in this study.

There were only three sets of twins (5.6%) observed throughout the study period and no cases of OHSS were reported. The rates of ART-conceived multiple births in the USA were reported to be above 40% in 2012 [47]. Furthermore, the medical costs were frightening, with ART singleton deliveries estimated at 26 922 USD versus 115 238 USD for ART twin deliveries (4 times) [48]. Similar findings were reported by Lukassen *et al.* [49]. There were no reported rates of multiple pregnancies in the South African ART registry report [9]. It is important to recognize that even in the low cost ART, a 26% rate of multiple pregnancies was reported and this is high [22]. This indicates and affirms that the individual consumer payment for ART not only influences access to treatment but also policies on the number of embryos to transfer [6]. Several studies have shown that in countries where governments are involved with public funding for ART and policy making, the benefits have been overwhelming. The programmes have shown a significant reduction in multiple pregnancy rates [3,17,50,51]. In poorly resourced countries with overburdened services, the negative outcomes of treatment should be avoided at all costs. It therefore makes it a primary goal of all involved with the care of healthy subfertile women to make it safe and minimize the financial burden of high order multiple pregnancies.

Provision of affordable ART services is certainly a worthy endeavour that should be supported and nurtured to increase access to treatment. This will further promote the Millennium Development Goals of achieving universal access to reproductive health by 2015 [52]. Beyond providing simple access to treatment, ART has been shown to reduce the risk of HIV transmission in serodiscordant partners. The number of people living with HIV worldwide continued to grow in 2010, reaching an estimated 35 million with approximately 2.7 million new infections [53]. According to the WHO, over half of people living with HIV are women, mostly of reproductive age, and they are the most vulnerable and marginalised when dealing with issues of infertility and procreation [54]. With evidence to show that ART can reduce the risk of contamination of the uninfected partner and help couples to conceive under safe circumstances, it thus further proposes a strong argument to make ART affordable and accessible in order to protect the uninfected partner in a heterosexual relationship [55-57]. The availability of ART service in the public hospital, particularly a teaching hospital, also provided a platform beyond just service delivery by allowing us the opportunity to continue to promote and improve teaching and training of reproductive medicine sub-specialists (currently four

in our programme), resident (registrars) rotating through infertility training and reproductive biology student, embryologist (currently two in training).

In conclusion, PPI as illustrated in this study, appeared to make ART accessible and allowed couples the opportunity to undergo treatment, therefore presenting a viable strategy towards ART provision in limited resource settings. In our opinion, this model can be reproduced and be implemented successfully in developing countries. However, further research should continue to evaluate and analyse the outcomes of other affordable methods of ART such as uterine-sperm-egg transfer [58], INVO Cell [59] and the tWE [60] in large clinical trials.

This full study, PPI model and description of patients treated is submitted for publication to Human Reproduction.

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Table 1: Patient demographic characteristics

Variables	Mean ± SD
Age (years)	34.9 ± 4.7
BMI	29.8 ± 2.4
Infertility diagnosis	
• Tubal factor	159 (45.6)
• Teratozoospermia	100 (28.6)
• PCOS	20 (5.7)
• Endometriosis	12 (3.4)
• Unexplained	52 (14.9)
• Others	6 (1.7)
BMI (kg/m ²)	

Table 2: Clinical outcomes

Variables	Available literature (local private data)	N (%)
Total number of 1 st cycle		237
Total number of 2 nd cycles		79
Total number of 3 rd cycles		37
<i>Total number of cycles performed</i>		375
Number of cycles reached oocyte retrieval stage		346 (92%)
Fertilization rate		251 (72.5%)
Mean number of oocytes retrieved		4.3 ± 4.0
Mean number of embryos transferred		2.99 ± 0.93
Mean number of good quality embryos on the day of transfer		
: day 2		2.09 ± 2.36
: day 3		2.02 ± 2.01
Average day of embryo transfer		1.27 ± 1.62
Cycles cancelled		150 (40%)
Reasons for cycles cancellation		
• No follicular development		29/375 (7.7%)
• No oocytes at aspiration		37/346 (10.7%)
• No fertilization		58/346 (16.7%)
• Poor quality embryo		26/346 (7.5%)

Table 3: Treatment outcomes

Variables:	N (%)	Data from SARA [9]
Clinical pregnancy/cycle	53/375 (14.1%)	
Clinical pregnancy/transfer	53/225 (23.5%)	
Clinical pregnancy/ET in women ≤ 35 yrs	29/114 (25.4%) *P=0.0041	584/1499 (40%)
Clinical pregnancy/ET in women >35 yrs	21/111 (18.9%) *P=0.01	390/1314 (29.6%)
Miscarriage rate	12/53 (22.6%)	
Ectopic pregnancy rate	1/53 (1.9%)	
Live birth rate/cycle	40/375 (10.6%)	
Live birth rate / ET	40/225 (17.7%)	
Multiple pregnancy rate:		
• Triplets	NIL	
• Twins	3/53 (5.6%)	
OHSS	NIL	
IVF	202 (53.8%)	
ICSI	167 (44.5%)	
IVF + ICSI	6 (1.6%)	

Table 4: Kaplan – Meier estimates of pregnancy per number of ART cycles

Cycles	Begin	Pregnancies	Cumulative Pregnancy rates	Std. Error	95% Conf. Int.	
Interval	Total					
	237	24	0.1412	0.0267	0.0969	0.2032
2	79	4	0.1984	0.0372	0.1363	0.2838
3	37	4	0.3172	0.0633	0.2110	0.4590
4	13	0	0.3172	0.0633	0.2110	0.4590
5	5	0	0.3172	0.0633	0.2110	0.4590
6	4	0	0.3172	0.0633	0.2110	0.4590

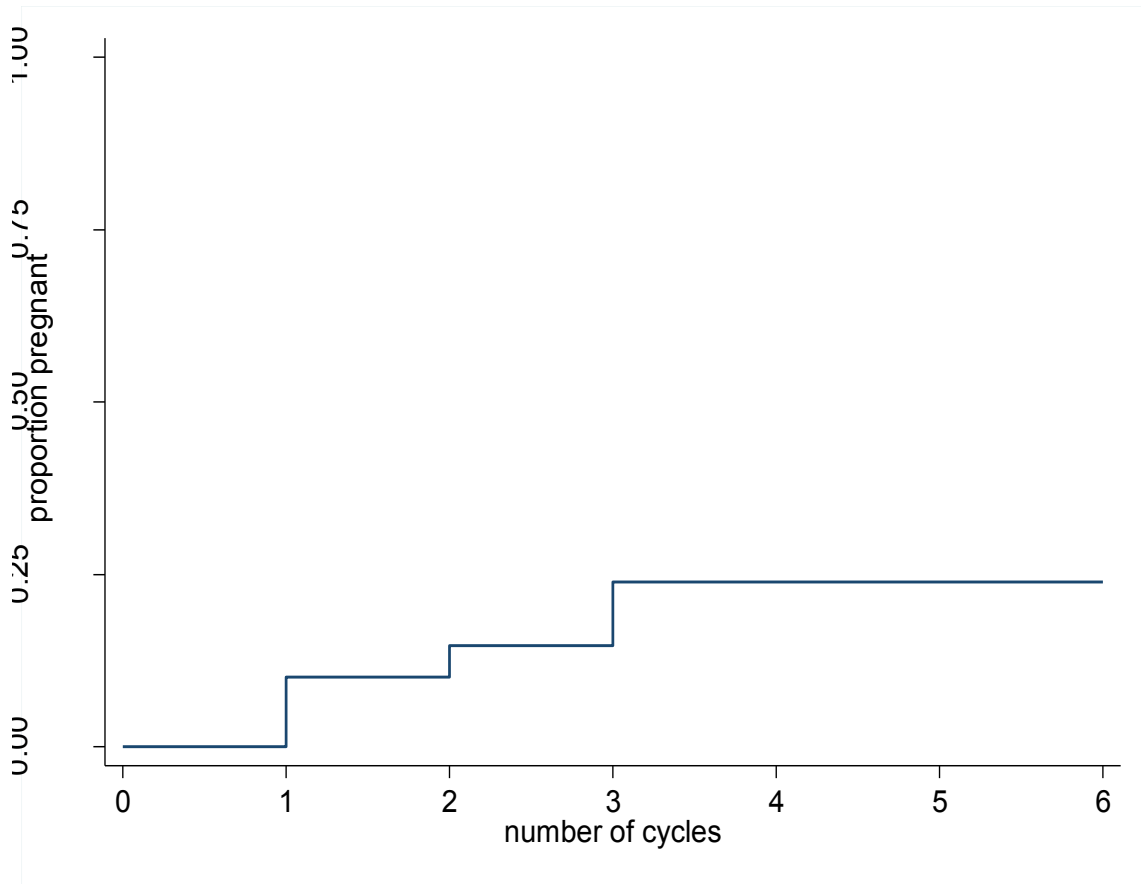
Laboratory costs + transfer		
Consumables including aspiration needles, Plastics, slides, pipettes, dishes, sperm prep	R363.03	R17 500.00 [8]**
ICSI pipettes.	R500.00	
*Aspiration needles + ET catheters	R927.92	
Cycle monitoring		
Ultrasound	R0.00	R1 610.00
Oestradiol + LH tests	R0.00	R400.00
Grand total	R121 326.00 - R167 326.00	R246 100.00 ^a
Direct cost to patient	R7 291 (Direct)	R51 000.00 (Direct cost to patient)
Subsidised:	R115 035 – 161 035	
Cost of IVF/ICSI cycle	R6 000 – 8 000	R35 000.00- R50 000 [8]***
Subsidised costs	R121 322 – 167 320	None

UPFS = Uniform Patient Fees Schedule, * Not always new, ** Direct costs to patients in 2012, there could be new figures, *** Price tag of IVF cycle which vary from clinic to clinic, ^a Estimated costs to company (clinic) based on one personnel in human resource categories (multiply by added number where applicable in big clinics). This cost is excluding medication and blood test fees.

Figure 1: Stimulation protocol

Stimulation protocol: Combination of Clomiphene Citrate and Menopur (75IU FSH& 75IU LH)										
Treatment day	1	2	3	4	5	6	7	8	9	
CC (100mg)			X	X	X	X	X			
Menopur(150-225IU)				X		X		X		
Ultrasound	Pre-treatment / AFC							X		
Urine LH									X	

Figure 2: Kaplan-Meier graph, probability of pregnancy per number of ART cycles



Addendum A: Signed Level of Agreement (SLA)

Provincial Government Western Cape - Department of Health - Tygerberg Hospital

SERVICE LEVEL AGREEMENT

Entered into between

TYGERBERG HOSPITAL

Represented by **Dr D. Erasmus**

in his capacity as Chief Executive Officer of *Tygerberg Hospital*

AND

AEVITAS CLINIC

Herein represented by **Prof Thinus Kruger**,
who warrants that he is duly authorised thereto

Background

This IVF service will be to the benefit of the community at large and with no significant cost to the State and Tygerberg Hospital. The programmes available in the private sector run at a cost between R25 000 to R35 000 per case and at Groote Schuur Hospital at R12 000 per case. This venture will render a cost effective service for patients in our region.

1. PURPOSE

To supply an in vitro fertilization service (test tube baby service) to patients in the Easter Metro pole as well as other patients in need for assisted reproductive technology (ART).

2. OBJECTIVES

- To render a low cost IVF service with minimal impact on the state resources to patients in need.
- To achieve universal access to reproductive health for patients in our region and those in need for fertility services in line with the United Nations Millennium Development Goals

3. PROCEDURE

3.1. Patients will be followed by an ART specialist with ultrasound and oocytes will be matured using the injection Ovitrelle.

3.2. IVF (test tube treatment) is done in the following way:

- Ovulation induction induced with CC and 3 ampoules of MMG
- Egg harvesting done under local block in the IVF laboratory.
- Embryo culture will take place in an incubator available in the IVF laboratory.
- Embryo transfer will be performed in the same area.
- With low cost IVF no ward or theatre will be used, saving a lot of cost.

4. SERVICE FREQUENCY AND STAFFING REQUIREMENTS

- The clinic will operate on a day to day basis
- 5-10 cases per month will be seen at the clinic
- Dr Thabo Matsaseng — University of Stellenbosch Scholarship and rendering a service to Tygerberg Hospital with permission of the authorities
- Dr Maria Viola - University of Stellenbosch Scholarship and rendering a service to Tygerberg Hospital with permission of the authorities.
- *Social Worker – To be arranged by Aevitas Clinic Staff if required.*

5. VENUE FOR THE PROVISION OF SERVICE

- Follow-up will happen at the ultrasound area, 3rd floor, Gynae outpatients.
- IVF treatment on the second floor in the existing IVF laboratory.

6. BILLING

- 6.1 For the infertility visit at Tygerberg outpatient department, patients will be billed by Tygerberg Hospital an amount equivalent of an OPD visit, in accordance to the Uniform Patient Fees Schedule (UPFS).
- 6.2 For the IVF service rendered, patients will directly be billed by the training unit of the US (Aevitas Clinic).

Currently the patients will pay an amount of R5000 to R8 500 per case, depended on the amount of medication used.

Category 1: Patients qualifying for full subsidization:

Ho = no payment

Category 2: Patients qualifying partial subsidization (H1, H2, H3)

H1=R35

H2=R120

H3=R194

Category 3: Full paying patients (No subsidy)

This category of patients includes but is not limited to externally funded patients. Non-South African citizens/Foreigners may be included. Full paying patients are liable for the full UPFS fee.

Tariff - Equivalent to a visit fee:

Private(P) - R241

Prescribed Minimum benefits(PMB/s): Infertility Code: 902M

Infertility is currently listed as a Prescribed Minimum Benefit (PMB) condition in the Medical Schemes Act 131 of 1998 under the category Female Reproductive System. Medical schemes are legally obliged to provide these Prescribed Minimum Benefits regardless of the member's package.

Pre-authorization/Authorization

All externally funded patients, i.e. confirmed medical scheme patients, including state departments patients attending public health institutions whereby the rule compels authorization / pre-authorization, shall ensure that the aforementioned is obtained. The onus is on the respective patient to obtain the authorization (pre-authorization) number, and is not the responsibility of the public health institutions to ensure the above.

7. CONSUMABLES AVAILABILITY

Aevitas Clinic, registered as a training facility of University Stellenbosch, will supply the following:

- Medication
- Medical Personal (ART specialists) to service to the patients
- Needles to harvest the eggs
- Growth medium for the embryos and semen preparation, plastic ware to use for embryo culture and semen preparation
- Embryo transfer catheters
- All other consumables

8. REVIEW OF THE CONTRACT

Neither the benefits nor the obligations under this SLA may be ceded or assigned by either party except with the prior consent of the other party.

The SLA will thus be reviewed every 3 or 6 months should the two parties deem it necessary.

9. INDEMNITY

The Service Provider agrees to hold Tygerberg Hospital harmless and indemnified, for the duration of this SLA, against all actions, litigations, demands, suits, proceedings (and all associated costs / expenses) which may be pursued against TBH arising from any real or perceived injury or damage or loss of property or death or injury caused to any persons during or as a result of the services agreed to in this SLA.

SIGNED at _____ on this ___ day of _____ 2007.

WITNESSES: _____

DR D. ERASMUS
Chief Director
Tygerberg hospital
Private Bag X1
TYGERBERG
7505

Service Provider

SIGNED at _____ on this ___ day of _____ 2007.

WITNESSES:

1. _____

PROF T. KRUGER
AEVITAS CLINIC/ US

CHAPTER 5: MILD OVARIAN STIMULATION FOR IN VITRO FERTILIZATION: ARE WE READY TO CHANGE? A META-ANALYSIS

SYNOPSIS

Objective: To compare the efficacy of mild ovarian stimulation versus conventional stimulation in IVF.

Design: Meta-analysis

Search strategy: A systemic literature search was carried out for prospective randomised clinical trials. We searched electronically using Pubmed, Medline and Embase for all studies published from 1990 to December 2011.

Interventions: Mild ovarian stimulation IVF, which uses lower doses and/or shorter duration of gonadotrophins in GnRH antagonist co-treated cycle compared with conventional stimulation IVF.

Main outcome measures: LBR per started cycle and OPR per started cycle of IVF

Results: A total of 148 patients per 769 cycles (70 mild and 78 conventional) showed a significant difference in favour of conventional ovarian stimulation for LBR [OR=0.59, CI: 0.41-0.85, P=0.004, Fig.1a]. Similar findings were observed in the ongoing pregnancy data of 284 patients per 1243 cycles (140 mild and 144 conventional) with significant difference in favour of conventional stimulation protocols [OR=0.72, CI: 0.55-0.93, P=0.01, Fig.1b]. The sub-analysis of two studies showed statistically significant reduction of hyperstimulation syndrome in favour of the mild stimulation [OR=0.27, CI: 0.11-0.66].

Conclusion: This analysis presents strong evidence in favour of conventional stimulation IVF and therefore should currently be considered a treatment of choice for patients requiring IVF treatment.

Keywords: IVF, ICSI, mild ovarian stimulation, conventional stimulation IVF, Clomiphene Citrate, live birth rate, ongoing pregnancy rate, treatment outcomes

INTRODUCTION

A recent publication by Sir Howard Jones reported on seven roads travelled to make assisted reproductive technology (ART) the success it is today, and suggested seven more roads to be travelled to continue to improve it [1]. Among them is to make ART safe, accessible and available to most infertile couples [1]. Furthermore it is a public health challenge to make ART available, affordable and accessible with minimal adverse effects without compromising its effectiveness.

Conventional ovarian stimulation protocol aims to provide the maximum number of oocytes retrieved for fertilization and thus several embryos for selection and transfer [2]. However, it is not without complications. The prevalence of potentially fatal severe OHSS is reported to be 0,5 – 5% [3]. Together with high order multiple gestation, OHSS remains a huge problem in ART treatments with significant morbidity and financial burden on health resources [4,5]. Pinborg *et al.* reported that 40% of children born following ART are twins, and these babies had a 7.4-fold increase in delivery before 32 weeks of gestation with significant increase in the admission to neonatal intensive care units [6]. The supra-physiological levels of steroid hormones seen in conventional ovarian stimulation are associated with adverse effects on endometrial receptivity [7] and may result in accentuated maturation of the endometrium, leading to embryo-endometrial asynchrony and reduced implantation rates [8]. Similar detrimental effects have also been associated with oocytes and embryo abnormalities [9,10]. The complexity of the long protocol, time consumption, patient discomfort, high costs and emotional distress are further associated with high drop-out rates from IVF / ICSI cycles [11-13].

The ideology that obtaining an increased number of oocytes leads to better pregnancy rates might be unjustified and contradictory [14]. Studies that evaluated the relationship between the number of oocytes retrieved and the pregnancy outcomes, reported an increase in pregnancy rates with a maximum of 15 eggs [15,16] and eventually a plateau or the decline in positive outcomes with an excess beyond this number of oocytes [16-18]. Furthermore, a recent meta-analysis suggests that the retrieval of a modest number of oocytes following mild stimulation is associated with higher implantation rates compared with patients where the same number of oocytes is retrieved following conventional stimulation [19]. Data suggest comparable outcomes between mild ovarian stimulation and conventional stimulation protocols but fewer complications, lower costs, and significantly fewer drop-outs

in mild protocols [11,12,20]. There is merit in considering these patient friendly approaches. To facilitate the acceptance and implementation of these strategies, we have decided to review all the published prospectively randomised papers that seek to evaluate the pregnancy outcomes in mild ovarian stimulation protocol versus conventional stimulation protocol.

Materials and Methods

Search strategy and identification of literature

A computerized literature search of all reports which described randomised controlled trials and prospective comparative trials of mild ovarian stimulation versus conventional stimulation was performed via Pubmed, Medline (1990 – 31 December 2011) and Embase (1990 – 31 December 2011). Relevant additional articles were hand-searched. A combination of medical subject headings (MeSH) and text words were used to generate subsets of citation such as 'mild ovarian stimulation', 'minimal ovarian stimulation', 'ovarian stimulation protocol', 'patient friendly strategies', 'soft stimulation', 'low ovarian response' and 'IVF or ICSI'. The menstrual disorders and subfertility group specialised register of controlled trials was also searched. The search was limited to trials in humans only. No language restrictions were placed on any search. The searches were conducted independently by TM and TFK. No written protocol of this review has been made or published.

Definitions

Mild stimulation in vitro fertilization

A mild cycle is defined as the method when FSH or HMG is administered at lower doses, and/or for a shorter duration in a GnRH antagonist co-treated cycle, or when oral compounds [anti-oestrogen or aromatase inhibitors] are used either alone or in combination with gonadotrophins with the aim of collecting a lower number of oocytes [21,22,23].

Conventional stimulation in vitro fertilization

This is defined as the term when a GnRH agonist is used for pituitary down-regulation followed by conventional doses of stimulation with FSH or HMG, or when a GnRH agonist is administered in a flare protocol with conventional doses of FSH or HMG, or when a GnRH antagonist is used with conventional doses of early start of FSH or HMG [21-23].

Study selection and data collection

Inclusion criteria

Studies were included if they reported the pregnancy outcomes in patients treated with mild ovarian stimulation compared to those treated with conventional stimulation protocols. All prospective randomised trials were included including those involving women below 38 years of age, regular menstrual cycle (25-35days), BMI of 18-29kg/m², normal hormonal profile and absence of uterine or ovarian abnormalities.

Exclusion criteria

Studies involving natural cycle conception, modified natural cycle conception, mild ovarian stimulation in a poor responder, oocyte donation, embryo donation and intra-uterine inseminations were excluded.

The following data collected from all the trials included: patient demographics, pattern of menstrual cycle, ovarian stimulation protocol, the number of oocytes retrieved, insemination technique [IVF/ICSI], fertilization rates, the number of embryos transferred, the clinical pregnancy rates, the ongoing pregnancy rates, and the live birth rates where available.

Outcome measures

The ideal primary outcome measure is the live birth rate. However, due to differences in the studies regarding the number of embryos transferred and the measure of their outcomes, the chosen primary outcome measure is the ongoing pregnancy rate. Other outcome measures such as clinical pregnancy rates, number of oocytes retrieved, number of embryos transferred, cycle cancellation rates, and complications such as OHSS were also studied and reported where possible. For the purpose of this review, clinical pregnancy rate was defined as the presence of the foetal heart beat on ultrasound at seven weeks of gestation, and the ongoing pregnancy rate was defined as the presence of the foetal heart beat on ultrasound at 12 weeks of gestation.

Data and statistical analysis

A score was allocated to each trial using the validated scoring system [24]. Six methodological variables such as randomization, group demographics, placebo use, follow-up, co-intervention and patient and cycle differentiation were chosen (Table1). Each trial was assessed and ranked for its methodological conduct and its potential to introduce bias. Trials were allocated scores that were divided by maximum possible and a percentage performance was given to each trial. Performance scores ranged from 79% to 100% (Table2). The data on the outcomes of each trial were summarized in two-by-two tables. The Peto odds ratio (OR) with its 95% confidence interval (CI) was calculated as the odds of an event in the mild stimulation group divided by the odds of an event in the conventional stimulation group in IVF/ICSI treatment. Statistical significance was inferred when the OR did not include 1.

The weight of each study in each analysis was calculated as inversely proportional to the variance. The degree of heterogeneity of studies was calculated using the chi-square test, with the p-value of <0.05 considered the limit of statistical significance, and I^2 statistics was used to describe the percentage of total variation across studies. An I^2 value of 0% was considered to signify no observed heterogeneity, while the values of 25, 50 and 75% were considered to indicate low, moderate and severe degrees of heterogeneity respectively. The applicable studies were re-analysed to find an explanation for any differences and also applied RevMan software to do a fixed effect meta-analysis. Analyses were performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA, 1999).

Results

Our search strategy identified 68 citations. Following the reading and scrutiny of the manuscripts, 43 studies were excluded by title and abstract and 20 did not meet the inclusion criteria by protocol. Therefore five studies met the inclusion criteria and were analysed. The characteristics of all the included studies are listed in Table 3. All five studies included were prospective and randomised. They were all approved by the local ethics committees.

The patient characteristics were comparable for all the studies, including regular indications for IVF/ICSI, age below 38 years, regular menstrual cycles (25-35 days) and absence of

severe endometriosis and uterine abnormalities. In these studies mild ovarian stimulation [21-23] was compared with conventional ovarian stimulation for IVF/ICSI.

The primary outcomes were live birth rates and ongoing pregnancy rates per cycle started. The secondary outcomes such as number of oocytes retrieved, total number of gonadotrophins used, cycle cancellation rates and OHSS rates were also reported.

Table 1: Validity criteria and scoring for methodology assessment of studies

Category	Score	Method
A Randomization	3	Randomized by central means [telephone and pharmacy] or sealed envelopes
	2	Alternating numbers
	1	Methods not described
B Group demographics	2	Demographics comparable
	1	Demographics not described
C Placebo use	2	Placebo or other treatment used in control group
	1	No placebo or other treatment
D Follow up	2	Outcome data for primary analysis complete
	1	Outcome data incomplete
E Co-intervention	2	Other than for use of treatment versus control, protocol involved same drugs
	1	Difference in protocols that may lead to contaminated results
F Patient and cycle differentiation	3	Only first treatment cycle included
	2	Patients included for more than 1 cycle
	1	Cycles and patients not differentiated

Table 2: Validity criteria score for each selected trial

Study	Score %	Randomization	Demographics	Placebo/ Other	Follow up	Co-intervention	Cycles
Baart et al, 2007	100	3	2	2	2	2	3
Blockeel et al, 2011	100	3	2	2	2	2	3
Heijnen et al, 2007	100	3	2	2	2	2	3
Hohmann et al, 2003	93	3	2	2	2	2	2
Karimzadeh et al, 2010	100	3	2	2	2	2	3

Table 3: Characteristics of included studies

Study	Design	Inclusion criteria	Study protocol [mild stimulation]	Control stimulation protocol[conventional]
Baart et al. 2007	RCT	Women below 38yrs of age, regular indication for IVF, regular menstrual cycle, BMI of 19-29kg/m ² , sperm count > 5mil/ml, no previous IVF cycle not resulting embryo transfer and no uterine or ovarian abnormalities.	Fixed 150IU/day of FSH from cycle day 5. GnRH antagonist 0.25mg/day was initiated when the leading follicle is 14mm	Long GnRH agonist for 2weeks and then fixed doses of 225IU/day of FSH
Blockeel et al. 2011	RCT	Regular indications for IVF, Age between 18-36yrs, BMI of 18-29kg/m ² , regular menstrual cycle, normal CD 2 FSH, no PCOS and no severe endometriosis.	Fixed 150IU/day of FSH from cycle day 5. GnRH antagonist 0.25mg/day was initiated on CD6	Fixed 150IU/day of FSH from cycle day 2. GnRH antagonist 0.25mg/day was initiated on CD6
Heijnen et al. 2007	Parallel group, non-inferiority randomised trial	Regular indication for IVF or ICSI, menstrual cycle length of 25-35days, BMI of 18-28kg/m ² , Age <38yrs, no previous IVF or healthy born child from IVF	Fixed 150IU/day of FSH from cycle day 5. GnRH antagonist 0.25mg/day was initiated when the leading follicle is 14mm. Combined with single embryo transfer.	Long GnRH agonist for 2weeks and then fixed doses of 150IU/day of FSH

Study	Design	Inclusion criteria	Study protocol [mild stimulation]	Control stimulation protocol[conventional]
Hohmann et al. 2003	Prospective randomised trial	Regular indication for IVF or ICSI, BMI of 19-29kg/m ² , Age 20-38yrs, regular menstrual cycle, no severe endometriosis or uterine or ovarian abnormalities, no more than three previous IVF cycles, no previous cycle with poor response or OHSS.	Fixed 150IU/day of FSH from cycle day 5. GnRH antagonist 0.25mg/day was initiated when the leading follicle is 14mm.	Long GnRH agonist for 2weeks and then fixed doses of 150IU/day of FSH or Fixed 150IU/day of FSH from cycle day 2. GnRH antagonist 0.25mg/day was initiated when the leading follicle is 14mm.
Karimzadeh et al. 2010	Prospective randomised controlled trial	First IVF attempt, Age 18-35yrs, BMI of 18-30kg/m ² , regular menstrual cycle length of 26-35days, basal FSH <10IU/L	CC 100mg/d from CD3 to CD7 Fixed 75IU/day of FSH from CD5. GnRH antagonist 0.25mg/day was initiated when the leading follicle is 12mm and additional 75IU/day of hMG to initial gonadotrophins	Long GnRH agonist for 2weeks and then flexible doses of 150 - 225IU/day of FSH

Table 4: Treatment outcomes of included studies

Study		Primary Outcomes		Secondary Outcomes			
		Live Birth Rates[LBR]*	Ongoing Pregnancy Rates[OPR]*	Number of oocytes retrieved†	Total number of gonadotrophins used per cycle§	Ovarian hyperstimulation syndrome [OHSS]*	Number of cycles cancelled*
Bart et al. 2007	Mild	NR	12[19]	8.3±4.7	NR	NR	NR
	Conv.	NR	7[17]	12.1±5.7	NR	NR	NR
Blockeel et al. 2011	Mild	NR	10[25]	10.3±6.2	1177±295¶	NR	NR
	Conv.	NR	10[27.7]	8.9±4.7	1364±226	NR	NR
Heijnen et al. 2007	Mild	70[15.8]	78[17.6]	6.9±4.8	1307±529¶	6[1.4]	80[18]
	Conv.	78[24]	93[28.6]	8.5±4.3	1832±758	12[4]	27[8]
Hohmann et al. 2003	Mild	NR	8[16.3]	7[1-27]‡	NR	NR	NR
	Conv.	NR	8[17.8]	9[1-25]	NR	NR	NR
Karimzadeh et al. 2010	Mild	NR	32[32]	5.4±1.5	12.1±4.3	0[0]	4[4]
	Conv.	NR	26[26]	9±2.2	22±3.6	6[6]	0[0]
Total	Mild	70[15.8]	140[20]	37.9±17.2	2496.1±828.3	6[2]	84[16]
Total	Conv.	78[24]	144[26]	47.5±16.9	3218±987.6	18[5.5]	27[9]

NR=not recorded, Conv=conventional, *data expressed as n [%], †data are expressed as mean ± standard deviation unless stated otherwise, ‡values expressed as median [range], §data expressed as mean number of ampoules ± standard deviation unless indicated, ¶ data expressed as mean total dose of rFSH [IU] ± standard deviation.

Primary Outcomes

Live Birth Rate

Although 'the singleton term gestation, live birth rate should be considered the best endpoint for ART [25,26], most studies still report live birth rates without being specific about the gestation, and a large proportion of them report the OPR only.

One study that reported live birth rates as an outcome showed statistically significant difference (70/444 [15.7%] mild versus 78/325 [24%] conventional cycles) in favour of conventional stimulation (OR=0.59, CI:0.41-0.85, P=0.004, Figure 1a). However, cumulative pregnancy outcomes were comparable (43.4% in the mild regimen versus 44.7% in the conventional regimen). It is the only study that recorded the cumulative pregnancy rates.

Ongoing Pregnancy Rate

Five studies with a total number of 284 patients also reported a statistically significant difference (140/696 [20%] mild versus 144/547 [26%] conventional cycles) in favour of conventional stimulation for OPR per started cycle (OR=0.72, CI: 0.55-0.93, P=0.01, Figure1b).

Figure 1a: Mild ovarian stimulation versus conventional stimulation: Live birth rates per fresh embryo transfer

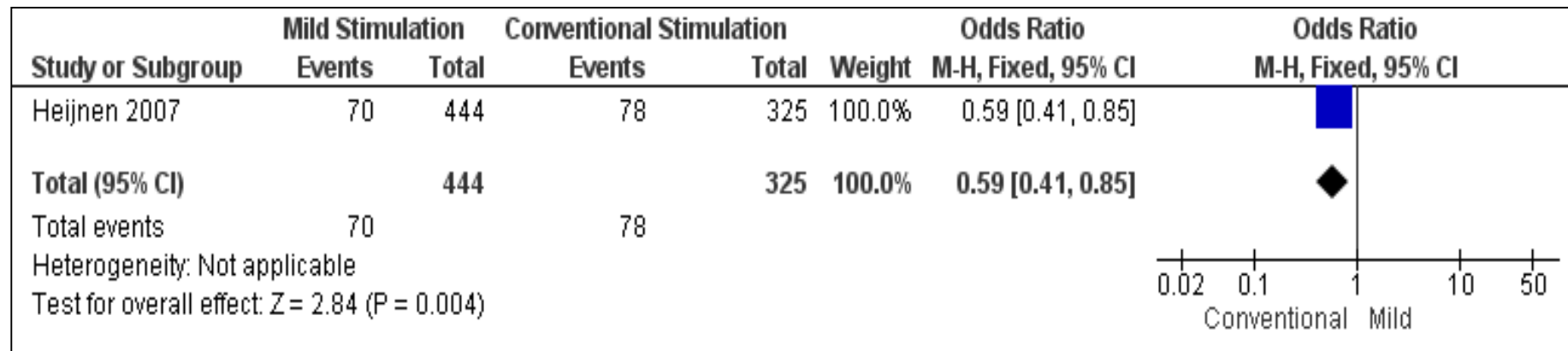


Figure 1b: Mild ovarian stimulation versus conventional stimulation: Ongoing pregnancy rates per fresh embryo transfer

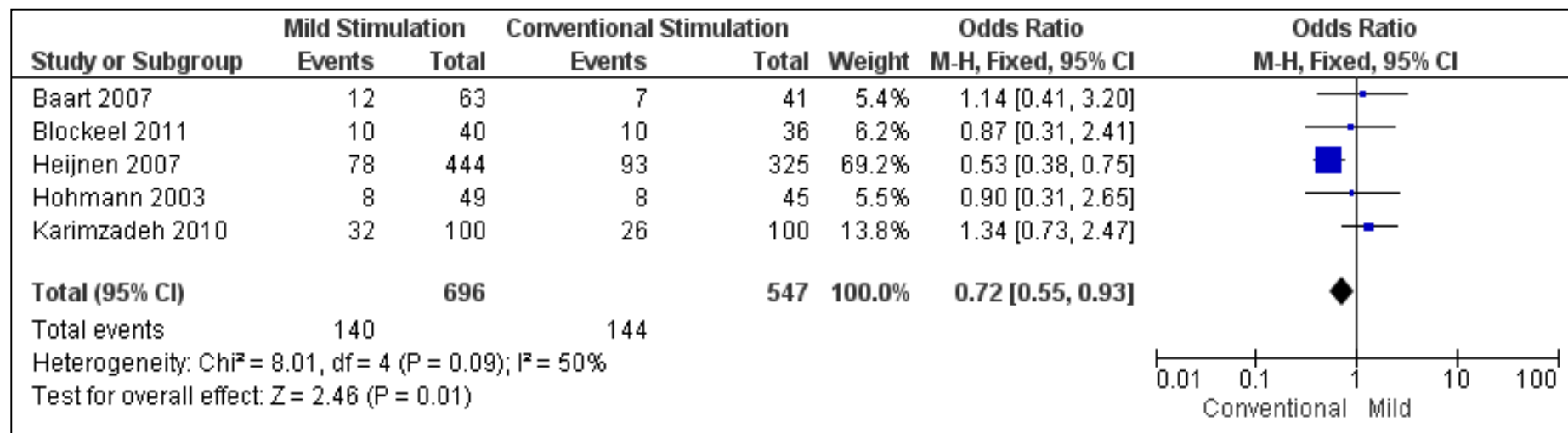
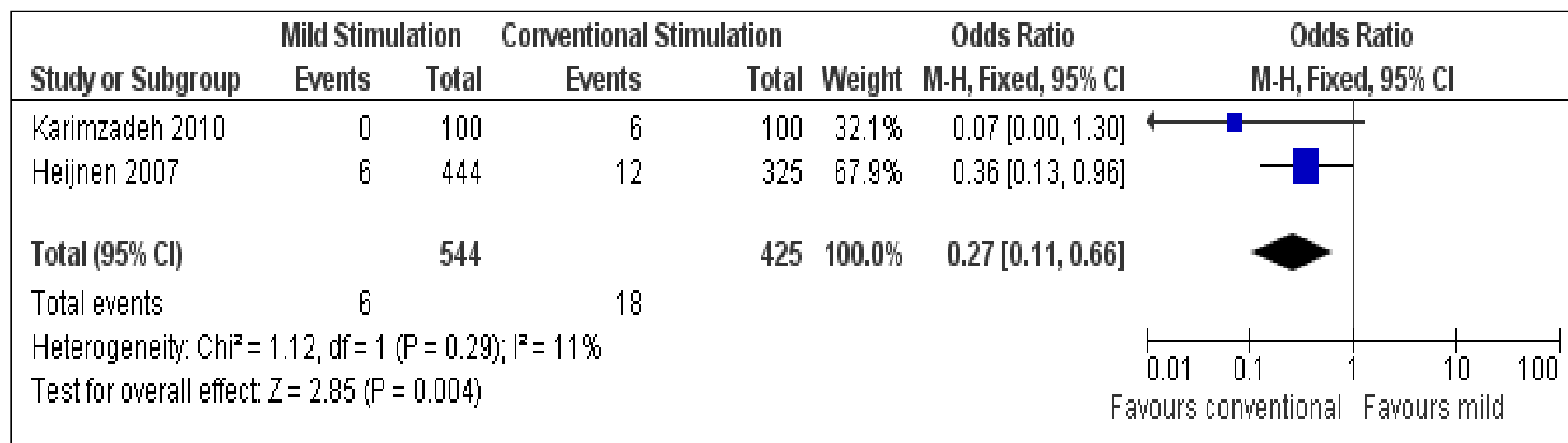


Figure 1c: Mild ovarian stimulation versus conventional stimulation: Ovarian hyperstimulation syndrome

Secondary Outcomes

Ovarian hyperstimulation syndrome

The sub-analysis of two studies showed a statistically significant reduction of hyperstimulation syndrome in favour of the mild stimulation (OR=0.27, CI: 0.11-0.66, P=0.0004, Figure 1c). No significant heterogeneity was detected between the studies.

Number of oocytes retrieved per cycle and total number of ampoules of gonadotrophins used per cycle

Analysed studies showed that a significantly lower number of eggs are retrieved in the mild stimulation protocols versus the conventional approach [37.9 ± 17.2 mild vs 47.5 ± 16.9 conventional protocols, P=0.000] and a significantly lower number of ampoules of gonadotrophins are used per cycle in the mild stimulation protocols (2496.1 ± 828.3 vs 3218 ± 987.6 conventional protocols, P=0.000) Table 4.

Number of cycles cancelled

The total number of cycles cancelled, including the poor follicular growth and failure to achieve embryo transfer, were significantly lower in the conventional protocol than in the mild [84 cycles in mild vs 27 cycles in conventional regimen, OR=2.55, CI: 1.62-4.02, P<0.0001].

Cost of treatment

Heijnen *et al.* also showed that the total cost of treatment is lower with mild protocol (difference €2412, 95% CI 703-4131) [20].

DISCUSSION

Over the past 30 years IVF treatment has improved, with recognizable developments in laboratory performance in terms of fertilization techniques, culture techniques for embryo development, embryo selection and cryopreservation of surplus embryos over and above improved ovarian stimulation protocols [27]. However, the introduction of mild stimulation protocols is still met with resistance in many units and the common reason is the lack of robust evidence to influence the current clinical practice in IVF. In this meta-analysis the conventional ovarian stimulation IVF showed significantly improved LBR and OPR when compared to mild stimulation regimens. The LBR per fresh ET from a study with a total

number of 148 patients (Table 4) significantly favoured conventional stimulation (OR=0.59, P=0.004) [20], therefore making it the treatment of choice in ART at this point.

It is interesting to note that data from the study by Heijnen *et al.* [20] showed no significant difference in the cumulative LBR in both regimens (43.4% in the mild regimen versus 44.7% in the conventional regimen). This observation underlines the need for more randomised controlled trials on cumulative live birth rate comparing the two regimens. The potential value of the mild regimen is a reduction of complication rate in ART. We observed a significantly lower risk of OHSS in the mild stimulation group as also reported by a number of authors, suggesting this regimen as a viable strategy for the prevention of OHSS [20,28,29]. In the current study, it was also observed that a significantly lower number of ampoules of gonadotrophins were used per cycle in the mild group compared to the conventional group and similar findings were showed by Blockeel *et al.* [30]. Polinder *et al.* reported significantly lower mean direct medical costs per IVF cycle for the mild regimen (€1559 versus €1977; P=0.001), mainly due to lower cost of medication [31]. A similar observation was reported by Heijnen *et al.* [20]. Due to its less complex nature, fewer ampoules of drugs and shorter duration used, it has been shown that mild stimulation IVF is also associated with a diminished level of patient distress [32]. The above facts propose mild stimulation as an attractive option for a low resource setting and selected group of patients at risk for OHSS.

Due to the lack of large randomised controlled trials, this meta-analysis identified and analysed data from prospective randomised trials conducted according to a reasonable standard that seek to add valuable information on the treatment outcomes in IVF stimulation protocols.

However a notable weakness in the papers is the lack of data on live birth rates, and the heterogeneity amongst the different studies [Figure 1b]. An attempt was made to minimize the heterogeneity by maintaining strict inclusion criteria in terms of patient profile and indications for ART [Table 1], adherence to the definition of mild ovarian stimulation [21-23] in comparison to conventional ovarian stimulation, and by analysing live birth rates and ongoing pregnancy rates since they would be affected equally in both arms.

In conclusion, this paper showed significantly better outcomes in terms of LBR and OPR per started cycle all in favour of conventional stimulation IVF, therefore currently remaining the

preferred treatment of choice. However, in the limited resource setting and in a well selected group of good prognosis patients, mild stimulation IVF may be considered a treatment option due to its potential benefits such as lower risk of OHSS, lower medication cost, less complexity in nature and lower levels of patient distress. In future more data on LBR in both mild and conventional stimulation IVF is still required for accurate scientific evaluation.

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CHAPTER 6: SUMMARY, CONCLUSION AND FUTURE RECOMMENDATIONS

The following summary and conclusion is based on extensive literature review and the findings of our studies, published and unpublished.

THE NEED FOR ASSISTED REPRODUCTION TECHNOLOGY IN DEVELOPING COUNTRY LIKE OURS WITH LIMITED RESOURCES

- Over 80 million couples are affected by infertility worldwide and the majority of them reside in the developing countries, where ART services are very scarce [1].
- Severe male infertility and bilateral tubal disease caused by sexually transmitted infections, unsafe abortions and postpartum sepsis are main reasons for infertility in developing countries [2].
- Couples are prepared to risk 20% of death to achieve the goal of parenting [3].
- Complicating the situation of inaccessibility to ART services in the developing countries, especially the sub-Saharan part of Africa, are the challenges around HIV and new infections.
- Serodiscordant partnerships account for up to 60% of new HIV infections. [4] Similar findings were reported by Makwe *et al.* [5]. Lurie *et al.* have shown that 30% of stable heterosexual couples in South Africa are HIV-1 serodiscordant [6] and 20-50% of these men and women do desire children [7].
- The dilemma of high risk behaviour motivated by this strong desire to reproduce becomes a realistic challenge, and it does exist [8,9].
- In 2010, the Minister of Health, Dr Aaron Motsoaledi, reaffirmed his commitment to the priorities of the National Department of Health Ten-Point plan to improve service delivery, and one of the Department's major targets was the reduction of new HIV infections by 50% by 2014 (target is missed but the battle must continue) [10].

- There were lost opportunities to focus on reduction of sexual HIV transmission. The emphasis was on prevention of mother-to-child transmission (PMTCT). Urging health care providers to actually communicate and preach safer conception strategies including early initiation of antiretroviral therapy and appropriate utilization of assisted reproductive technologies, did not receive the same emphasis [11,12].
- There is only 6% coverage of ART needs in South Africa (SA) because ART is an expensive treatment, unaffordable and still very much inaccessible to many [13]. A cycle of IVF in private clinics in South Africa will cost the individual couple approximately R35 000 - R50 000 (2 702-3 861 USD) [14] and the cost drivers are mainly medication (28%), clinicians' fees and consultation (29%), and laboratory fees (35%) [14]. The cost of an IVF cycle is also reported to account for 52% of an individual couple's average disposable income [15].

Based on the above-mentioned facts and knowledge of the cost drivers in IVF, the objective was to develop a model that can focus on lowering the cost of treatment and make it accessible to more patients. This is how the thesis with the theme "Low cost IVF Strategies" was conceived.

The robust literature search and the findings of the thesis highlighted the following aspects to consider when establishing affordable ART:

Understanding the physiology of folliculogenesis - Chapter 1

Of the three theories of follicular recruitment, continuous recruitment which suggests that small antral follicles of $\leq 4-6$ mm are recruited to grow continuously at all stages of reproductive life independent of gonadotropin support [16], might substantiate the mild ovarian stimulation regimen rationale of late introduction of gonadotropins in IVF/ICSI cycle and discontinuation a day or two prior to HCG administration in non-pituitary suppression cycles – thereby significantly lowering the total amount of gonadotropins required and hence the cost of medication. Furthermore, the duration of the rise in FSH above the critical threshold determines the number of dominant follicles selected from the recruited cohort. This phenomenon is termed the "FSH Threshold" or "FSH Window" or "FSH gate" [17]. During ovarian stimulation therapy, prolonging the FSH window allows multiple follicles to

be selected, illustrating again that it might not necessarily be about the quantity of gonadotropins required but the length of exposure. This is a very important concept in mild ovarian stimulation, especially in good prognosis patients (normal endocrine profile and women of young age, < 35yrs).

Establishing the role of inexpensive and safe oral medication, Clomiphene Citrate as the ovulation induction agent in assisted reproductive technology - Chapter 2

Clinical pregnancy rates in IVF cycles of normal responders using CC alone could vary from 16% per started cycle to 34% per ET [18-21]. In women with poor ovarian response or compromised ovarian reserve, the use of CC alone in IVF has been associated with pregnancy rates of 5-10% [22,23]. Although the rates were low, they were comparable to those of women treated with conventional long GnRH agonist protocol and large amounts of gonadotropins [23], thereby making CC alone IVF a cheap reasonable option for women with depleted ovarian reserve, both young and old. The introduction of gonadotropins, either alone or in combination with oral agents, was introduced in IVF with the aim to achieve an increased number of oocytes for fertilization [24]. Subsequently, several studies using combination therapy reported acceptable clinical pregnancy rates per ET of 32-43% [25-29]. However this treatment regime is reported to be associated with high rates of premature LH surge (approximately 27%) and significantly high cancellation rates, ranging between 15-30% [30,31]. Our randomised controlled trial in Chapter 3 of this thesis (below) reported similar findings of high premature LH surge in CC plus gonadotropin ART treatment. The effective method of preventing premature LH surge in ART cycles is pituitary gonadotropin suppression using a GnRH agonist (long, exhausting and expensive) or GnRH antagonist (short and relatively cheap) as explained in Chapter 2. In poor resource settings these methods will remain unaffordable because they require an increased number of gonadotropins at a high cost. Proposed, less expensive but effective methods are published in the literature, but the limitations of the studies were old data, small sample sizes and heterogeneous data with different outcomes. They include pre-treatment with oral contraceptives and prolonged use of CC during ovarian stimulation [19,32]. We therefore performed a randomised controlled trial to evaluate the use of prolonged CC as a method of preventing premature LH surge in ART programmes (protocol outlined below). In addition, progesterone (Medroxyprogesterone acetate, Utrogestan) and antiprogestin (Mifepristone) have also been proposed as effective oral alternatives for the prevention of premature LH

surge in women undergoing controlled ovarian hyperstimulation [33-35]. This is based largely on animal model studies which demonstrated that progesterone (P) successfully blocks the E₂-induced LH surge in both follicular and early luteal phase of rhesus monkeys and ewes when started early in the follicular phase of the menstrual cycle [36,37]. It is worth mentioning that in all progesterone studies the endometrial thickness was not critically analysed during the stimulation protocol and the embryos were cryopreserved and subsequently followed by frozen thawed embryo transfers, therefore calling for further evaluation of this protocol with a closer look at the endometrium and outcomes if fresh embryo transfer is to be performed.

A simple method of an extended 8-day course of Clomiphene Citrate versus a 5-day course in an attempt to suppress premature luteinizing hormone surge in an assisted reproductive technology programme: a randomized controlled trial - Chapter 3

The purpose of the study was to evaluate the simplified and inexpensive method of preventing premature LH surge in ART treatment.

Treatment protocol

The patients were randomised into one of two treatment groups using a simple randomization schedule assigned via numbered sealed envelopes following patient assessment.

In Group A, the patients were stimulated with CC (Fertomid, Cipla MEDPRO RSA) 100mg for five days from cycle day 3 to 7. hMG (Menopur, FERRING, SA) 150-225IU was added on cycle day 4, 6, 8 and/or 10.

In Group B, patients were stimulated with CC (Fertomid, Cipla MEDPRO RSA) 100mg for eight days from cycle day 3 to 10. hMG (Menopur, FERRING SA) was added on cycle day 4, 6, 8 and/or 10. No baseline endocrine biochemical testing was performed in either group. Ultrasound was performed in both groups on cycle day three for AFC and evaluation for any cyst formation. The subsequent ultrasound was performed on cycle day 8 or 9 and thereafter according to follicular growth. LH was assessed from cycle day 9 and if negative, performed on alternative days until the day of hCG administration (Ovitrelle 250µg/0.5ml, Merck SA). If

LH positive, the oocyte retrieval would be performed within the next 24 hours. hCG (Ovitrelle, Merck SA) 250µg/0.5ml was administered when there was a leading follicle of ≥ 18 mm.

In this study we found the rates of premature LH surge to be high with 20% (5-day Clomiphene group) versus 24% (8-day Clomiphene group). The cycle cancellation rates were also high, 36% (5-day CC group) vs 31% (8-day CC group). The main reason for cancellation was failed embryo transfers as a result of poor embryo development and growth, which may strongly suggest a negative impact of premature LH surge on the quality of oocytes. However, cycle cancellation as a result of poor follicular development occurred in 10% (5-day CC arm) and 11% (8-day CC arm) of the cases. Our study could not replicate the findings of previous studies looking at a low dose CC of 50mg as a strategy to prevent premature LH surge in IVF/ICSI cycles. Perhaps the populations studied were different and the methodology also differed. We were not concerned that, not prolonging the duration of CC further by a day or two until the day of hCG trigger as suggested in previous studies, would affect the anti-oestrogenic properties of the drug on the pituitary and lose the benefit of lowering the LH and subsequent prevention of LH surge. More so, it is unclear how long it thus takes for CC (enclomiphene & zuclomiphene) to metabolise and clear from the system. Our study concluded that prolonged use of CC was not associated with a reduction of premature LH surge in mild ovarian stimulation ART programmes, therefore recommending other affordable methods of suppressing premature LH surge such as pre-treatment with a oral contraceptive pill [19] and the use of progesterone and FET cycles [33-35] to be explored and evaluated in large clinical trials. In addition, the need to further evaluate the use of low dose CC until the day of hCG [38] or the use of tamoxifen at a dose of 40mg from cycle day 3 until the day of hCG (with positive stimulating effect on endometrium when compared to CC) [39] with or without the use of non-steroidal anti-inflammatory drugs such as indomethacin to prevent spontaneous follicular rupture [40], will still be necessary in larger studies.

During the trial we observed a clinical pregnancy rate per ET and LBR per ET of 24% and 16% respectively. We believed the outcomes were acceptable for a mild stimulation protocol but wanted to know at what cost and whether the model we were using could be a solution towards making ART accessible to sub-fertile couples in resource restricted settings. This question led to the description and evaluation of the PPI model outlined below.

Description and implementation of the Public-Private Interaction model - Chapter 4

This is a structured model that began with the willingness and commitment of the administration of Tygerberg Hospital and Stellenbosch University (public sector) and the caring and generous involvement of the Drs Aevitas Institute for Reproductive Medicine (private sector) with an altruistic intention to help (Addendum A). This is the vision that tertiary institutions must have, to establish strong and positive relations with private facilities for the benefit of patients or communities (service delivery) and students at large (research, training and teaching).

- **Infrastructure** development can be made possible by the public sector. This is a significant and costly component of ART services. The minimum amount of space required is approximately a two-bedroom area of 120 m² to provide adequate service. This small area of space can be identified within the hospital building, ideally close to areas with easy access to resuscitation facilities such as an operating theatre. Andrology services can be integrated within the IVF set-up.
- **Equipment:** Essential equipment for basic ART services includes: (Addendum B)
 - Incubator
The equipment was acquired through the Stellenbosch University grant. This is a notable contribution by the university as they understood our mandate to continue research, teaching and training of sub-specialist fellows, registrars, honours and masters reproductive biology students.
 - ICSI micromanipulator
The machine was donated by the Drs Aevitas private clinic, which was a significant contribution considering the current cost of the equipment (approximately R500 000).

Contributions of such magnitude lay a strong foundation in the establishment of specialised services such as IVF/ICSI treatment in the public sector services.

- **Personnel:** Competent staff, both clinicians and embryologists, are the essential components of successful ART services [41]. Staff remuneration contributes 30% to the cost of IVF in the private setting [14]. In the PPI model, the clinicians and the embryologists are employed either by the provincial government or by the university, primarily for service delivery and research, training and teaching respectively. They therefore incorporate extended ART services within the day-to-day routine of their duties. In this model, there are no IVF sisters or co-ordinators, which makes the model cheaper but adds a lot of strain on the clinicians and the scientists alike, who have to assume those responsibilities. Under current circumstances and limited resources the system is functional and can be sustained.
- **Stimulation protocol:** A mild ovarian stimulation protocol was adopted in this model as another strategy to lower the cost of IVF/ICSI treatment. It is defined as the method when FSH or HMG is administered at lower doses, and/or for a shorter duration in a GnRH antagonist co-treated cycle, or when oral compounds (anti-oestrogen or aromatase inhibitors) are used either alone or in combination with gonadotrophins with the aim to collect a smaller number of oocytes [42-44]. The anti-oestrogens include CC and/or tamoxifen.

We used CC because it is cheap, well studied and because of its safety profile. We have observed reasonable pregnancy outcomes in CC IVF cycles without the use of mid-cycle antagonists. (Mentioned and discussed in detail in Chapter 2.)

The decision to use hMG, Menopur in the treatment protocol was based on the drug's effectiveness and relatively low cost in comparison to other gonadotropins [45].

With regard to cycle monitoring, it is cheaper and safe to use the ultrasound and urinary LH test only without adding serum biochemical tests because there is no proven significance of the additional tests in terms of improvement in IVF outcomes [46,47]. In a mild ovarian stimulation the risk of hyperstimulation is very low [44] and further supporting less intense monitoring.

- **Laboratory:** Further illustration of the PPI benefit is in the laboratory section of ART services:
 - Large private clinics purchase media, plastics and other consumables in bulk and the small units like Tygerberg unit (public) access these consumables at a reasonably lower price (manufacturer's price) from them.
 - The aspiration needles and embryo transfer catheters received from the private clinic following a single usage are sterilized according to medical devices sterilisation and safety standards [48] and re-used to further lower the costs of treatment. Following the once-only repeat use of these devices, they were then discarded. The sterilization of medical equipment is generally an accepted practice also seen in operating theatres [48].
 - The egg retrieval is performed under conscious sedation and local anaesthesia, without the need for general anaesthesia and theatre, further reducing the cost of treatment significantly. In a limited resource setting, the method does not interfere with other services because it does not utilize or remove the hospital's anaesthetist or nursing sisters from their assigned duties. The medication is administered by the clinician performing the procedure and there is always a supporting clinician on standby if necessary. The duties of the nursing sister are performed by the embryologist in the laboratory.

We have observed CPR per ET and LBR per ET of 23.5% and 17.7% respectively which are low when compared to conventional IVF outcomes (SARA report) [13]. The study has also shown that in women ≤ 35 yrs of age the clinical pregnancy rates per ET were 25.4% vs 40% of conventional IVF data from the South African Registry of Assisted Reproduction Technologies, SARA (12 IVF clinics contributed to the registry) [13]. Women > 35 yrs of age had pregnancy rates also significantly in favour of conventional IVF with 29.6% vs 18.9% from our study [13]. These findings were also observed by the meta-analysis we performed (Chapter 5) [49], which presents strong evidence in favour of conventional stimulation IVF and therefore suggests that it be considered a treatment of choice for patients requiring IVF treatment. The challenge is the cost of single IVF/ICSI treatment [14]. In our analysis (Chapter IV, Table 5) we established that the direct cost of one conventional IVF to a couple is approximately R51 000 (USD 3 938) vs R7 291 (USD 563) in the private vs public sector respectively. We also observed that the probability of better outcomes (CPR $> 30\%$) in a low

cost model will require a repeated number of treatment cycles (at least three attempts). What is not clear in the SARA report was the number of individual cycles required to achieve the reported pregnancy rates [13].

Another notable finding of the study (Chapter 4, Table 4) was to show that 237 participants started the first cycle of treatment but only 37 participants were able to attempt the third cycle of treatment. The main reason for the significant decline was lack of funds. This gave us an impression that the model gave access to many couples who would have not have had any opportunity to attempt parenthood, but left a big question mark as to whether the treatment is affordable or not. – further proposing an economical model in our setting to assess at what cost ART can be declared affordable. This is an analysis which we plan to undertake in collaboration with the Health Economics Division of the Department of Health.

In conclusion, the PPI between Tygerberg Academic Hospital, Stellenbosch University (public entities) and Drs Aevitas Institute for Reproductive Medicine (private facility) is a model that has made ART services more available to the infertile community of the Western Cape. Because of its relatively low cost, it has attracted patients from all over South Africa and Sub-Saharan Africa (Malawi & Zambia) who are desperate to be parents but cannot afford the private fees. We believe the model has achieved the primary goal of making ART accessible based on the number of requests we receive on a daily basis from both HIV negative and positive patients. We aim to promote the model to other institutions in South Africa and Africa as a whole and hopefully to reduce the burden of the disease, improve access to treatment and offer overall improvement in quality of life of people living with infertility in the continent. For a central academic hospital embarking on ART services but limited by resources, a qualified reproductive specialist and embryologist are mandatory to begin with. Basic infrastructure is essential (small room within the hospital environment). Regarding equipment, mobilization of the private sector could be a valuable exercise to purchase a basic incubator and a microscope, and the service can then be rendered (page 152, Figure 3). However there is also ongoing research to explore the effectiveness of simple embryo culture systems such as the INVO cell or the walking egg tWE system (page 102). The centres can also be supported through outreach programmes.

Overall, this thesis has demonstrated that there is a role for minimal ovarian stimulation protocols (Literature review in Chapter 2 & RCT in Chapter 3) together with other cost saving

strategies (Descriptive PPI study in Chapter 4) for people living in low income countries with limited resources. However, there must be an effort and innovative strategies to further improve pregnancy outcomes per cycle of IVF/ICSI treatment started. As shown by the findings of our meta-analysis (Chapter 5) that pregnancy rates per cycle are still significantly higher with conventional IVF, patients with associated endocrine abnormalities such as PCOS, or those with endometriosis, should be advised appropriately regarding the outcomes of different regimens.

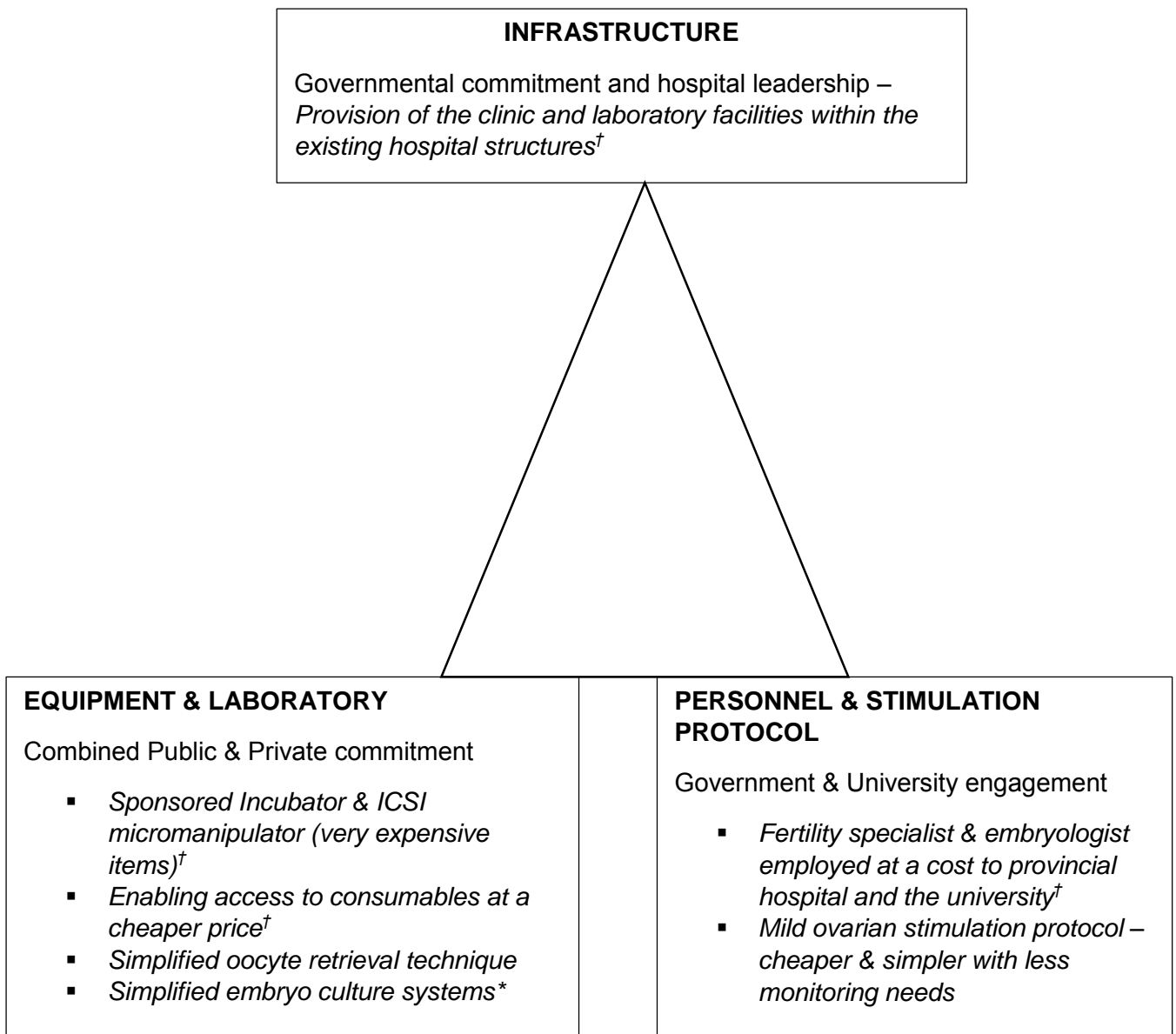
Ethically and generally speaking, the couples should be provided with options and adequate information regarding the outcomes of ART treatments in order to make an informed decision. In that way the aim to provide equal access to good quality health care could be achieved.

FUTURE RECOMMENDATIONS

- PPI is a feasible model to improve the provision of health care in resource-restricted settings – it should be pursued and implemented where necessary.
- Commitment, vision and political willingness are fundamental pillars towards improving access to health care and ART in particular for the benefit of improving access to fertility treatment.
- Mild ovarian stimulation protocols should be encouraged especially in good prognosis patients and in limited-resource countries.
- However, the effort through research should continue to identify strategies that optimise the outcomes in these regimes by means of acceptable pregnancy rates, lowering the risk of premature LH surge and cycle cancellations. The methods should be effective and affordable as well.
- Investing in competent personnel is also instrumental towards successful implementation of health services in general and also specialised services such as ART. The clinicians and embryologist must form part of the broader base of the hospital staff members, thereby providing services other than ART.
- Further laboratory strategies that aim to reduce the costs of IVF treatment and improve access may include the “The Walking Egg” (tWE) project [50] and the INVO cell devices [51]. However the methods need to be evaluated in large clinical trials. We are embarking on participating in a multi-centre trial to answer some of these questions.

- Other methods of affordable IVF such as uterine-sperm and egg transfer (U-SET) should also be evaluated in large clinical trials [52].
- Figure 3 below illustrates the three basic pillars of implementing IVF services and the impact of the PPI model in facilitating and enabling the process.

Figure 3: Basic IVF Implementation triangle illustrating the PPI model†



*The Walking Egg project (tWE) using simplified embryo culture system and the INVO Cell device still to be evaluated in large multi-centre clinical trials and hopefully be integrated as part of routine method of treatment in any simplified IVF/ICSI cycle (both in developing and developed countries).

It is important and necessary that the private sector at large, not necessarily the ART clinics, should be given an opportunity to participate and get involved with community building projects and projects that seek to facilitate academic development and strengthening through corporate social initiatives.

www.youtube.com/watch?v=V0ts4aC-GsY

www.bbc.co.uk/news/health-27256416

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Addendum B: Basic IVF/ICSI equipment

- CO₂ Incubator
- Microscope – ordinary
- Laminar flow
- Refrigerator
- Centrifuge
- Media preparations
- Cryopreservation systems (simple freezing method)
- Micromanipulator
- Clean room (filters, positive pressure, stainless steel equipment)
- Heated stage
- Necessary consumables (oocyte retrieval, sperm preparation, insemination techniques, fertilization media, embryo culture media, and embryo transfer).