A descriptive study on the use of a gonadotropin releasing hormone agonist ovulation trigger in assisted reproductive techniques

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DECLARATION

I, Yosef Yitchok Unterslak, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Obstetrics and Gynaecology in the University of the Witwatersrand, Johannesburg.it has also been submitted to the Colleges of Medicine of South Africa for partial fulfilment of the requirements for the qualification of Fellowship of the College of Obstetrics and Gynaecology.

Yosef Y Unterslak

16 February 2015

DEDICATION

I dedicate this work to my late father Dr R.L. Unterslak of blessed memory. My father was not only the greatest clinician I ever learnt from but more importantly my father taught me how to have true empathy for my patients. My father never cried for his patients, he cried with them, feeling the physical and emotional pain that his patients were suffering. His service in this world was to be a facilitator of G-d in healing. Unfortunately, he himself could not be healed. I hope to be able to emulate the work my father did and that I can make him proud with my service to the profession of medicine.

To my wife Ester and amazing children, thank you for your patience during my endless years of study. Your support and love are what has gotten me this far.

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PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

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2. Presented as an oral presentation at the 1st FIGO Africa Regional Conference in Obstetrics and Gynaecology – 2-5 October 2013, Addis Ababa, Ethiopia

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LIST OF ABBREVIATIONS

AMH	**Anti-Mullerian Hormone**
ARDS	**Acute respiratory distress syndrome**
ART	**Assisted reproductive technique**
COH	**Controlled ovarian hyper-stimulation**
D	**Day**
E2	**Estradiol**
FSH	**Follicle stimulating hormone**
GnRH	**Gonadotropin releasing hormone**
GIFT	**Gamete intrafallopian transfer**
hCG	**Human chorionic gonadotropin**
ICSI	**Intra-cytoplasmic sperm injection**
IQR	**Intra-quartile range**
IVF	**In-vitro fertilisation**
LH	**Luteinizing hormone**
OHSS	**Ovarian hyper-stimulation syndrome**
PCOS	**Polycystic ovarian syndrome**
RCT	**Randomised control trial**
ZIFT	**Zygote intrafallopian transfer**

ABSTRACT

Background and Objectives

The risk of developing ovarian hyperstimulation syndrome post-induction of ovulation in patients undergoing in-vitro fertilisation has been greatly reduced by the introduction of gonadotropin-releasing hormone agonists for ovulation induction. The pregnancy outcomes have not been fully evaluated and specifically not when fresh embryo transfer takes place on day three or day five of the medicated cycle as opposed to frozen embryo transfer in a fresh non-medicated cycle.

The objectives of this study were:

1. To evaluate the incidence of moderate to severe ovarian hyper-stimulation in patients undergoing gonadotropin-releasing hormone agonist induced ovulation

2. To evaluate the pregnancy rates achieved when gonadotropin-releasing hormone agonist induced ovulation takes place and embryos are transferred fresh as opposed to frozen

3. To assess parameters such as stimulation drug used, pre-trigger oestrogen values and pre-treatment anti-Mullerian hormone and evaluate them with respect to pregnancy outcomes in order to isolate the best candidates for gonadotropin releasing hormone agonist induced ovulation induction

Methods

This was a descriptive study done at the Vitalab Centre for Assisted Conception. All patients undergoing in-vitro fertilisation and placed on the Cetrotide® -

[cetrorelix acetate (gonadotropin-releasing hormone antagonist)] between the months of April 2010 through to April 2011 were used for the study. These patients had undergone ovulation induction with Lucrin® - [Leuprorelin acetate (gonadotropin releasing hormone agonist)]. Patients younger than 18 and older than 35 were excluded along with those defined as poor responders to in-vitro fertilisation.

Results

Forty eight of the 59 patients had more than 18 leading follicles at stimulation. The mean leading follicle size was 16.8mm. The interquartile range for pre-trigger estradiol was 12575 – 23672pmol/L with a mean of 18337pmol/L. Seven of the 59 subjects were coasted and only one patient developed moderate ovarian hyperstimulation syndrome. Only 2 patients had zero oocytes collected while four patients did not have embryo transfers. The biochemical pregnancy rate was 39% at 14 day post embryo transfer and the clinical pregnancy rate was 34% at the seven week ultrasound.

Conclusion

Gonadotropin-releasing hormone agonist ovulation trigger almost entirely eliminates the risk of ovarian hyperstimulation syndrome and when fresh embryo transfer takes place it results in biochemical and clinical pregnancy rates which are acceptable. The use of intensive luteal phase support needs further work to establish whether the pregnancy rate would be so high without it.

1. Introduction

For a couple struggling to conceive, assisted reproductive techniques (ART) including in-vitro fertilisation (IVF) offers them some hope of one day joining the ranks of parenthood. A study by Domar et al done across four European countries showed that women undergoing fertility treatment felt more hopeful and closer to their husbands than those women struggling to conceive but not going for any treatment.[1] As much as IVF offers these couples the chance to conceive, it comes with a tremendous burden on couples who have to undergo the treatment. IVF is an expensive procedure for which many couples save money for months or even years. The average cost of an uncomplicated IVF cycle in Johannesburg, South Africa is roughly R35 000[2]. IVF also confers a significant burden on one's family, and social life, work performance and emotional wellbeing. A recent study by Pinto-Gouveia et al looked at one hundred women with known infertility compared with one hundred couples that did not have fertility problems. The study found that the couples struggling with infertility exhibited higher scores on depression and lower scores on acceptance and self-compassion, as compared to the control group.[3]

2. Literature review

2.1. History of IVF

The first child born from IVF conception was Louise Brown, born in Oldham, England in July 1978 after work done by Robert Edwards and Patrick Steptoe.[4]

An Australian clinic, Monash IVF, achieved the first pregnancy in a woman without ovaries in 1983 after using donor eggs and a special hormonal formula to support the first ten weeks of pregnancy.[5]

The first pregnancy following intracytoplasmic sperm injection (ICSI) took place in 1992.[6] Since then reproductive medicine has developed to an extent that we now have the ability to screen for genetic defects in an embryo before it is transferred to the uterus, thus eliminating certain genetic diseases in a child or population[7] .

2.2. Normal ovulation and fertilisation[8]

To understand the role of medicated fertility cycles, it is important to appreciate normal ovarian function. This shall be explained briefly in the following description.

Ovulation typically takes place under tight control of the hypothalamic-pituitary-ovarian axis. Pulsatile productions of gonadotropin-releasing hormones (GnRH) by the hypothalamus stimulate the pituitary to produce gonadotropins namely: follicle stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropins in turn stimulate the ovaries to produce progesterone and oestrogen which act with a negative feedback on the pituitary causing it to produce a smaller amount of gonadotropins. The first day of the menstruation is known as day (D) 1. There is a significant rise in the FSH levels during the first four days of the cycle stimulating the development of a primary follicle in the ovary. The primary follicles undergo specific changes until one of them becomes the dominant follicle. Only one

primary follicle usually becomes the dominant follicle and this selection depends on the number of FSH and oestrogen receptors produced on the dominant follicle. The primary follicles and then later the dominant follicle produce oestrogen which; stimulates the development of a glandular proliferative endometrium, turns the cervical mucus into a medium receptive to sperm and acts with positive and negative feedback on the pituitary to either increase or decrease the production of FSH and LH. The oestrogen level rises and when it reaches approximately 300pg/ml at roughly D12-D13 of the cycle, it causes an LH surge which in turn impacts on the dominant follicle, now known as the pre-ovulatory follicle, for ovulation. LH promotes luteinisation of the granulosa cells in the dominant follicle resulting in the production of progesterone. Ovulation occurs roughly 10-12 hours after the LH surge. The progesterone level in the follicle continues to rise causing a negative feedback and a termination of the LH surge. Progesterone then causes an increase in permeability to water, of the follicle wall, rapidly increasing the follicular fluid volume. Changes in the collagen of the follicular wall make it thinner and cause it to stretch. In the presence of prostaglandins which cause contractions of the smooth muscle cells of the ovary, ovulation takes place. Post ovulation, the granulosa cells are luteinised and organise to form the corpus luteum which begins secreting progesterone peaking roughly on D9 post ovulation. The corpus luteum rapidly declines after nine to eleven days post ovulation unless ovulation results in a pregnancy during which human chorionic gonadotropin (hCG) will be secreted and the corpus luteum will survive.[8]

2.3. In-vitro fertilisation

In–vitro fertilisation is the process where an egg is fertilised by a sperm outside of the body. IVF is an assisted reproductive technique during which the woman

undergoes a heavily medicated and manipulated cycle stimulating the ovaries to produce multiple dominant follicles with the aid of drugs such as synthetic FSH known as controlled ovarian hyper-stimulation (COH). There are two protocols currently described for IVF namely the “long protocol” and the “short protocol”.

During the long protocol, the pituitary-ovarian axis is down-regulated by the administration of a gonadotropin-releasing hormone (GnRH) agonist. The ovaries are then hyper-stimulated by the administration of injectable gonadotropins - FSH analogues. The administration of these FSH analogues usually begins 10-14 days after the initiation of the down regulation of the pituitary-ovarian axis. Down-regulation of the pituitary-ovarian axis is necessary to assist the physician in preventing premature luteinisation - it prevents the natural process of ovulation taking place. Since high doses of gonadotropins are administrated to hyper-stimulate the ovaries, the physician requires full control of the timing of ovulation, as it must coincide with the leading follicles being of the correct size and number.

The short protocol omits the down regulation of the pituitary-ovarian axis and FSH analogues are used to encourage follicular development during a natural cycle. In a short protocol ovulation is suppressed by the administration of a GnRH antagonist towards the end of the follicular phase of the cycle. During both the long and short protocols, the response of the ovaries is monitored by the use of transvaginal ultrasound and oestrogen levels. Ultrasound monitoring indicates when there are 2 or more leading follicles measuring more than 17mm each. Once this is seen, ovulation induction can take place.

Ovulation induction is defined as the stimulation of final oocyte maturation and ovulation by means of the administration of either synthetic human-chorionic gonadotropin (hCG) or more recently a gonadotropin-releasing hormone agonist.[9]

Ovulation induction is akin to a synthetic LH surge and can be achieved by the administration of a low dose of hCG resulting in final oocyte maturation and ovulation roughly 36-48 hours later.[10] Ovulation induction may also be achieved by the administration of a GnRH-agonist resulting in a more natural LH surge.[11] Once ovulation induction has taken place, transvaginal oocyte retrieval can take place. Under ultrasound guidance, one end of a hollow needle is inserted transvaginally into the ovary. The other end is attached to a suction device and gentle suction is applied to aspirate the follicular fluid and oocytes. The procedure is repeated for both ovaries and takes place under conscious sedation, general anaesthesia or regional anaesthesia[12, 13]. Once the oocytes have been collected, oocyte and sperm preparation can take place. The oocytes are identified and stripped of surrounding cells to be prepared for fertilisation. Semen is prepared from the ejaculate by removing inactive cells and seminal fluid in a process known as "sperm washing". Sperm may be washed by means of density gradient centrifugation or by the "direct swim-up" method.[14] The sperm and oocytes are then incubated in a culture medium at a ratio of about 75000 sperm to 1 oocyte.[15] Typically embryos are cultured until they reach a 6-8 cell stage roughly day three post fertilisation although they may be grown in an extended medium till day 5. Embryo transfer takes place either on D3 or D5, or can be done using frozen-thawed embryos and is done as a conscious procedure under ultrasound guidance. The embryos judged to be the best based on the embryologist's opinion are loaded into a plastic catheter which is passed through the cervix and are expelled from the catheter high into the uterus by the aid of a syringe. The number of embryos transferred will depend on the amount available, the choice of the physician and patient, and the circumstances of the patient.

2.4. Ovarian hyper-stimulation syndrome (OHSS)

With the process described above, one can imagine the added stress on a couple and health care provider when an IVF cycle needs to be cancelled. There are many reasons for having to cancel an IVF cycle with ovarian hyper-stimulation syndrome (OHSS) being one of them. In a study by Aljawoan et al looking at a group of patients undergoing IVF/ICSI with more than 20 follicles at the time of ovulation induction, 10.4% of those that had three leading follicles greater than 15mm and a serum estradiol level greater than 1635pg/ml (±6000pmol/L) developed moderate to severe OHSS.[16]

OHSS is almost exclusively iatrogenic and is caused by the administration of synthetic hCG used to trigger final oocyte maturation prior to oocyte collection.[17] The incidence of OHSS has been estimated to occur in 1% to 10% of cases of IVF and occurs as a serious life threatening condition in 0.1% to 2% of assisted reproductive cycles.[18] OHSS is a systemic disease resulting from vasoactive products released by hyper-stimulated ovaries. OHSS is characterised by increased capillary permeability leading to leakage of fluid from the vascular compartment leading to third space fluid accumulation and intravascular dehydration.[19] OHSS results in a massive fluid shift resulting in an accumulation of up to 17 litres of fluid in the peritoneal cavity. This fluid collection results in organ dysfunction, respiratory and circulatory failure.[20]

According to the latest classification by Golan, OHSS is divided into mild, moderate, severe and critical OHSS.[21]

- Mild OHSS: characterised by abdominal swelling, mild abdominal pain and ovarian size less than 8cm.

- Moderate OHSS: characterised by moderate abdominal pain, nausea and vomiting, ultrasound evidence of ascites and ovarian size between 8 and 12cm.
- Severe OHSS: includes clinical ascites, oliguria, haematocrit above 45%, hypoproteinaemia and ovarian size greater than 12cm.
- Patients with critical OHSS need intensive care admission. These patients present with tense ascites, pleural effusions, haematocrit above 55%, white cell counts above 25000cells/ml, oliguria or anuria, thromboembolism related to OHSS and acute respiratory distress syndrome (ARDS).

Moderate to severe OHSS has been estimated to occur in 0.2% to 2% of ovarian stimulation cycles.[22] OHSS can be further divided into early onset and late onset OHSS. Early onset OHSS occurs in the luteal phase and is directly linked to the administration of exogenous hCG. Late onset OHSS occurs when the treatment results in a pregnancy and is as a result of endogenous hCG following conception.[23]

As with all medical conditions, prevention is better than cure and thus profiling high- risk patients that are at risk of developing OHSS is a crucial step in fertility treatment. Risk factors for OHSS have been identified and categorised as primary and secondary risk factors.

Primary risk factors include:

- young age (less than 33 years old),
- polycystic ovarian syndrome (PCOS)
- previous OHSS.[24]

Secondary risk factors are indicated by ovarian response to the stimulation and are assessed during the stimulation phase and include:

- high number of medium and large sized follicles generally greater than 20 follicles all less than 14mm
- high or rapidly rising E2 levels above 3000pg/ml
- number of oocytes retrieved.[25]

2.5. Prevention of OHSS

Once primary or secondary risk factors have been identified, numerous options are available to the clinician to prevent OHSS from developing. A recent publication suggests that individualized controlled ovarian stimulation is the way to prevent OHSS. Some measures available include cycle cancelation, coasting; the use of a GnRH-agonist in place of recombinant hCG for final oocyte maturation, individualisation of the hCG trigger and cryopreserving all embryos for subsequent transfer in an un-stimulated cycle.[26] Cycle cancellation can be both financially and emotionally crippling to a patient and is reserved as a last resort in cases where OHSS may be severe or critical. Coasting is used to decrease the level of serum estradiol by withholding the exogenous gonadotropins while still administering the GnRH-antagonist. This method reduces the number of granulosa cells on the dominant follicles and in turn reduces the amount of circulating estradiol. Withholding of exogenous FSH causes accelerated apoptosis of granulosa cells and atresia of the smaller follicles and thereby reduces the number of follicles.[27] Individualisation of the hCG trigger still poses a threat of both early and late onset OHSS.

Previously ovulation induction took place with the administration of synthetic hCG. More recently, to prevent OHSS, newer agents such as gonadotropin-releasing hormone(GnRH) agonists have been used to induce ovulation.[28] In order to use a GnRH-agonist to induce ovulation, a GnRH-antagonist must be used to prevent the premature LH surge and uncontrolled ovulation. Administration of a GnRH-agonist induces an LH surge from the pituitary similar to the spontaneous mid-cycle LH surge. Because GnRH-agonists have been used to desensitize the pituitary during the standard IVF cycles, it has not been widely used as a means to trigger final oocyte maturation. Since the introduction of GnRH-antagonists to prevent the premature LH surge, the use GnRH-agonists for final oocyte maturation and ovulation induction is now possible.[29] A 2011 Cochrane review of the use of a GnRH-antagonist to prevent premature LH surge compared with GnRH-agonist showed a reduction in the incidence of moderate to severe OHSS while live birth rate and on-going pregnancy rates were comparable with a 95% confidence.[30] Manzanares et al showed that triggering ovulation with a GnRH-agonist followed by embryo cryopreservation allows patients with polycystic ovaries to complete COH in vitro fertilisation (IVF) without any cycle cancellation, coasting or OHSS. Manzanares also found the pregnancy outcomes to be comparable to non-GnRH-agonist trigger cycles such as recombinant hCG.[31] In the abovementioned study the clinical pregnancy rate was 33%.[28] This study looked at a protocol in which embryos were frozen and then transferred in a fresh non-medicated cycle. Originally, the thinking was that embryos derived from a cycle in which a GnRH-agonist was used for ovulation induction, must be frozen and transferred a month later in a natural non-medicated cycle. The reasoning behind this was that the very short endogenous LH surge caused a subsequent

defective corpus luteum and in turn impacted on the implantation of the embryo and resulted in lower pregnancy rates.[32]

The reason for the comparable pregnancy rates in the study by Manzanares was because the embryos were frozen and transferred a month later in a non-medicated cycle.

In a systematic review and meta-analysis, Griesinger et al looked at the use of a GnRH-agonist to trigger final oocyte maturation in a GnRH-antagonist, ovarian stimulation protocol. In these studies the embryos were not cryopreserved and were transferred in a medicated cycle. Twenty three publications were identified of which three fulfilled the inclusion criteria for the meta-analysis. The likelihood of achieving a clinical pregnancy when embryos were not frozen was found to be considerably reduced with an odds ratio of 0.22, (95% CI = 0.05-0.85, p=0.03).[33]

A 2010 Cochrane review by Youssef et al looked at 11 randomised control trials (RCT)s of which eight studies assessed fresh autologous cycles. In fresh, non-donor cycles, GnRH-agonist was less effective than hCG in terms of the live birth rate per randomised woman (OR 0.44, 95% CI 0.29 - 0.68; 4 RCTs) and on-going pregnancy rate per randomised woman (OR 0.45, 95% CI 0.31 - 0.65; 8 RCTs). This study proposed that for a group of patients with a 30% chance of live birth rate using hCG, GnRH would reduce the chances to between 12% and 20%. Moderate to severe OHSS incidence was significantly lower in the GnRH-agonist group compared to the hCG group (OR 0.10, 95% CI 0.01 - 0.82; 5 RCTs). From this study the authors concluded that GnRH-agonists should not be used for final oocyte maturation when fresh embryo transfer was taking place. The authors did however recommend the use of GnRH-agonists in the population that posed a very high risk of developing moderate to severe OHSS but advised to warn the

patients of the potential risk of cycle failure.[34] A small prospective, observational study looked at a “freeze all” policy when GnRH-agonists were used for final oocyte maturation in patients at high risk for OHSS. This study found a cumulative on-going pregnancy rate of 36.8% and an on-going pregnancy rate per first frozen-thawed embryo transfer to be 31.6%.[35]

Because of the above studies, the worldwide consensus has been that when a GnRH-agonist is used to trigger final oocyte maturation, the resultant embryos are then frozen on D3 or D5 and then thawed and transferred in a non-medicated natural cycle one month or some months later as opposed to the medicated cycle during which the oocytes are extracted.[31] The pregnancy rates following frozen-thawed embryos have always been lower than fresh embryo transfer.[36] Frozen-thawed embryo transfer has been found to have a cost benefit as one can retrieve numerous oocytes per cycle, fertilise them, and then after transferring one or two of the resultant embryos the remainder can be frozen and subsequent IVF cycles can be shorter and cheaper. However, the place for frozen-thawed embryo transfer should be reserved for cases of cost saving and should not ideally be used as a first line method in ART.[36]

Certain methods have been described in an attempt to improve pregnancy outcomes when a GnRH-agonist is used to trigger final oocyte maturation. One such method is the freeze all method described by Devroey et al. and supported by Griesinger et al.[37, 38] in the “freeze all” method, oocytes are collected and fertilised and all embryos are frozen and transferred in a non-medicated cycle. Other suggestions to improve the pregnancy rate post- induction of ovulation with a GnRH-agonist, include the use of a dual trigger of both GnRH-agonist and low dose hCG with intensive luteal phase support or intensive luteal phase support

alone.[39, 40] The optimal luteal phase support is still undecided amongst clinicians, with protocols differing from clinic to clinic. A 2011 Cochrane review attempted to define the optimum luteal phase support by reviewing randomised controlled trials of luteal phase support in assisted reproductive techniques. This review investigated the use of progesterone, hCG or GnRH-agonist supplementation in in vitro fertilisation or intracytoplasmic sperm injection cycles. It also analysed different combinations of progesterone and oestrogen, different preparations and routes of administration of progesterone as well as combinations of progesterone with either hCG or GnRH-agonists. The authors concluded that progesterone seemed to be the best option for luteal phase support, with synthetic progesterone showing better results than micronized progesterone. The review showed benefit to adding a GnRH-agonist to progesterone when looking at live birth, clinical pregnancy and on-going pregnancy. Of particular interest to the reviewers was the significant increase in OHSS when hCG was used either alone or in combination with progesterone and the reviewers recommended avoiding hCG entirely. As much as this review seems to favour progesterone alone, the reviewers did caution against the results as the number of studies in each comparison was small and there was a high risk of type 2 error in most included studies.[41]

3. Problem statement

The risk of developing OHSS post-induction of ovulation in patients undergoing IVF has been greatly reduced by the introduction of GnRH-agonists for ovulation induction. The pregnancy outcomes have not been fully evaluated and specifically not when fresh embryo transfer takes place on day three or day five of the medicated cycle as opposed to frozen embryo transfer in a fresh non-medicated cycle. Vitalab Centre for Assisted Conception performs IVF cycles using a GnRH-agonist to trigger final oocyte maturation and transfers the resultant embryos in the same medicated cycle from which the oocytes were extracted. As this is an unconventional method the researcher felt it necessary to study the patients that have undergone GnRH-agonist-trigger IVF and to determine the pregnancy outcomes and incidence of OHSS.

The aim of this study was to evaluate the pregnancy rates achieved in GnRH-agonist ovulation induction IVF cycles and to evaluate the rate of moderate to severe OHSS in this group. Our aim was to show that there was no improvement in pregnancy outcomes when embryos are frozen and transferred in a non-medicated cycle as is the current practice while practically eliminating the risk of OHSS.

4. Objectives

1..To evaluate the incidence of moderate to severe OHSS in patients undergoing GnRH-agonist induced ovulation (Mild OHSS is seen in almost all cases of ovarian stimulation for IVF and thus only cases of moderate, severe or critical will be assessed)

2..To evaluate the pregnancy rates achieved when GnRH-agonist-induced ovulation takes place and embryos are not frozen

3..To assess parameters such as stimulation drug used, pre-trigger oestrogen values and pre-treatment AMH and evaluate them with regards to pregnancy outcomes in order to isolate the best candidates for GnRH-agonist induced ovulation induction.

5. Methods

5.1 Setting

The Vitalab Centre for Assisted Conception was the setting for this study. Vitalab is a leading fertility clinic in South Africa. Drs' Jacobson, Gobetz and Volschenk are registered Reproductive Medicine Specialists with the Health Professions Council of South Africa and have a combined experience in excess of 50 years in evaluating and treating infertile couples.

5.2 Study design

This was a descriptive study retrospectively analysing the pregnancy outcomes of a defined group of patients undergoing final oocyte maturation and ovulation induction with a GnRH-agonist.

5.3 Study Population

The records of all patients undergoing IVF and placed on the Cetrotide® - [cetrorelix acetate (GnRH antagonist)] protocol that underwent ovulation induction with Lucrin® - [Leuprorelin acetate (GnRH-agonist)] between the months of April 2010 to December 2011 were included in the study.

5.3.1 Inclusion criteria

The records of patients between the ages of 18 and 35 years old undergoing IVF on the Cetrotride® protocol between April 2010 and December 2011 were considered for the study.

5.3.2 Exclusion Criteria

Patients found to have poor ovarian reserve were excluded. Poor ovarian reserve was based on serum FSH being greater than 10U/L on day three, age greater than 35 years old and low anti-mullerian hormone (AMH) of less than 1.1ng/ml.[42,43]

The reason that poor responders were excluded from the study was that these patients are not expected to develop OHSS. When a patient is assessed to be at risk of being a poor responder, the patient will automatically be put onto a long stimulation protocol with Lucrin downregulation and will therefore not be a candidate for Lucrin trigger.

5.3.3 Protocol:

The following is a summary of the protocol used by Vitalab when GnRH-antagonist stimulation is used.

On day three of the cycle the patient presents to the clinic for a baseline scan and a blood test to determine the serum estradiol (E2) and progesterone levels. At this point both the male and female start taking antibiotics and antifungals. The current protocol uses Ciproflaxacin 500mg twice daily for five days and Fluconazole 150mg once off as antibiotic and antifungal therapy as per the Vitalab protocol based on international data. Recent studies have shown an improvement in pregnancy outcome in patients with repeated IVF failure after the use of the antibiotics and antifungals mentioned. One hypothesis is that the reason for pregnancy failure could be a benign intrauterine infection.[44] The day of the scan becomes known as D1 of the stimulation. On day 1 of the stimulation either Gonal-f® [(follitropin alfa made by Merck)] or Menopur® [(menotropins made by Ferring)]

is started. These drugs are both forms of exogenous FSH. The dosage and drug used depends on the patient's prior response, antral follicle count, age, AMH and body mass index (BMI) and is calculated based on the current recommended protocols as stipulated in the Journal of Fertility and Sterility. This is continued until day 6 of the stimulation when a ultrasound scan is done and an E2 level is checked. Based on the scan and E2 level Cetrotide® – GnRH antagonist is started on either D6 or D7 and its role is to suppress the natural LH surge. At this point both Cetrotide® and either Gonal-f® or Menopur® is being administered daily. The patient will then be scanned either daily or every second to third day depending on the scan findings and E2 levels until there are at least 2 leading follicles of 17mm or greater in size each. It is at this point when the drug of choice to trigger final oocyte maturation and ovulation will be decided upon. This decision is based on the number of follicles and the E2 levels at the time of the trigger. If the patient has more than 18 leading follicles or the estradiol levels are greater than 18000pmol/L (±4900pg/ml) the patient will undergo a GnRH-agonist trigger for final oocyte maturation and ovulation. Patients not showing signs of being at high risk of developing OHSS either based on the number of follicles or the E2 levels will then be given Ovidrel as the ovulation induction agent. E2 and progesterone levels are checked pre-trigger and post trigger. Roughly 36 hours post trigger transvaginal oocyte collection takes place in theatre under conscious sedation.

Embryo transfer and luteal phase support post GnRH-agonist trigger

Patients that have undergone GnRH-agonist trigger receive Gestone® (intramuscular progesterone preparation) 100mg injection intramuscular daily along with Estro-Pause® (oral estradiol valerate) 2mg tablets twice daily and

Estraderm TTS patch® (transdermal estradiol system) replaced every second day. These patients are also on Folic Acid 5mg daily from before the cycle and start Ecotrin® (enteric coated acetylsalicylic acid) 81mg daily on the day of embryo transfer. These patients will also have a quantitative βhCG on D14 if the embryo/s had been transferred on D3 or D12 if the embryo/s had been transferred on D5.

Clinical pregnancy is defined as a positive fetal heart on transvaginal ultrasound at 7 weeks post embryo transfer. A biochemical pregnancy is defined as a positive serum βhCG on day 14 or day 12 post embryo transfer depending on which day embryo transfer took place.

5.4 Sampling and Sample size

All relevant files during the predefined study period were retrieved and evaluated. A total number of 59 files were reviewed and included in the study.

All patients undergoing ART on the Cetrotide protocol except those fulfilling the exclusion criteria were included in the study.

5.5 Data collection

The data were collected by the researcher. All cases of assisted reproduction have been recorded in a register at the Vitalab Centre for Assisted Conception. With the assistance of the filing clerk, all patients that underwent IVF on the Cetrotide® (GnRH antagonist) protocol during the predetermined study period were isolated and their files drawn. Patients were then selected according to the inclusion and exclusion criteria set out in the study protocol. Those cases eligible for the study were assigned a patient number which can only be traced back to the file by the researcher thereby ensuring anonymity of the patients. All relevant data were

recorded on a data sheet, an example of which is attached. The data were then translated into an Excel spread sheet for analysis.

5.6 Data analysis

Once all the data had been collected and transferred onto a spread sheet, the data were analysed with the assistance of a statistician. Descriptive data were described using means with standard deviations and modes with ranges. Analytical statistics comprised of Student's T test for comparisons of means and the Chi-squared test for comparisons of frequencies.

5.7 Ethics

Total anonymity of the patients included in the study was maintained throughout. Ethical clearance was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. (Appendix)

6. Results

6.1 Demographics and pregnancy history

During the collection period a total of 59 patients satisfied the inclusion criteria described above. Of the 59 cases reviewed, the age of the patients ranged between 20 and 35 with the mean age being 30.55 (SD 3.3).

Table 1: Range of age of patients in the study

Age	Frequency	Percentage (%)
20-25	4	7
26-30	23	39
31-35	32	54

Fifty one out of the 59 patients had never had a viable pregnancy resulting in a primary infertility rate of 86%. Of the 8 patients that had live births previously, 6 had had one child and 2 had two children.

Table 2: Gravidity of the patients included in the study

Gravidity	Frequency	Percentage %
0	35	59
1	17	29
2	2	3
3	3	5
4	2	3

Seventeen of the patients had experienced at least one miscarriage. Two of the patients had sustained ectopic pregnancies previously.

Table 3: Incidence of miscarriage amongst the patients on the study

Miscarriages	Frequency	Percentage %
0	42	71
1	11	19
2	4	7
3	1	2
4	1	2

6.2 Pre-treatment work up

A pre-treatment antral-follicle count was performed on all patients. Patients with more than 12 antral follicles were said to have “polycystic-like ovaries”.

Table 4: Ranges of antral follicle counts in the right ovary of all patients included in the study

Antral follicle count – right (n)	Frequency	Percentage %
0-3	7	12
4-6	15	25
7-9	9	15
10-12	5	8
>12	23	39

Table 5: Ranges of antral follicle counts in the left ovary of all patients included in the study

Antral follicle count – left (n)	Frequency	Percentage %
0-3	9	15
4-6	11	19
7-9	7	12
10-12	7	12
>12	25	42

The antral follicle counts were recorded for right and left ovary as opposed to a total number as this is the convention in which the antral follicles are counted at

the clinic where the study was conducted. This is because some patients may have a poor antral follicle counts on one side and when stimulated one does not become alarmed when a good response is seen on only one of the sides. One of the patients did not have a left ovary due to previous pelvic surgery. The patient with only one ovary had an antral follicle count of 5 in the right ovary.

The pre-treatment AMH levels ranged from 1.6ng/ml to 48.5ng/ml with an interquartile range (IQR) of 2.9ng/ml to 8.7ng/ml and a median of 5.4ng/ml.

Pre-treatment FSH had in IQR of 5.5U/L to 7.8U/L and a median of 6.4U/L.

6.3 Cycle specific data

The mean D2 E2 level was 124pmol/L with an IQR of 71pmol/L to 183pmol/L and the mean D2 progesterone level was 1.4pmol/L.

The most commonly used drug for stimulation was Menopur® with second most popular choice being a combination of Menopur® and Gonal-F®. Fifteen percent of the patients were stimulated with Gonal-F® alone.

Table 6: Stimulation drug used

Drug	Number of patients (n)	Percentage %
Menopur®	31	53
Gonal-F®	9	15
Combination of above drugs	19	32

The average stimulation duration was 9.1 days (SD±1.5) The shortest stimulation took 4 days and the longest a total of 14 days.

Forty eight (81%) of the 59 patients had more than 18 leading follicles at stimulation. The average leading follicle size was 16.8mm with a standard deviation of 1.4.

Table 7: Size of leading follicles

Size of Leading Follicles (mm)	**Frequency**	**Percentage %**
13	1	1.7
14	1	1.7
15	8	30.6
16	17	29
17	13	22
18	13	22
19	5	18.5
20	1	1.7

The IQR for the pre-trigger E2 levels ranged between 12 575pmol/L and 23 672pmol/L, with a median of 19 552pmol/L. The lowest pre-trigger E2 was 740pmol/L and the highest was 43 493pmol/L.

Seven (12%) of the 59 subjects were coasted. Only one patient developed moderate ovarian hyper stimulation syndrome.

The mean number of oocytes collected was 12.5 with two patients having no oocytes collected, and the greatest number of oocytes collected totalling 28.

Table 8: Number of Oocytes Collected

Number of Oocytes Collected	Frequency	Percentage %
0-5	5	8
6-10	12	20
11-15	28	48
16-20	11	19
>20	3	5

6.4 Choice of artificial reproduction technique

Thirteen of the 59 patients underwent ICSI alone. Thirty seven of the 59 patients underwent IVF. Six of the patients had both ICSI and IVF performed, a further 2 patients had zygote intra-fallopian tube (ZIFT) and 1 patient had gamete intra-fallopian tube (GIFT).

6.5 Fertilisation results

Fertilisation was deemed successful when two pronuclei were observed. Four of the patients did not have oocytes fertilised. One of the patients had 20 oocytes fertilised. The most frequent number of oocytes fertilised was 9.

6.6 Embryo transfer

Four patients did not have embryo transfer performed while 25 of the 59 had embryo transfer on day 5. Twenty-seven of the 59 had embryo transfer on day 3. Of the 4 patients that had no embryo transfer; 1 had 8 oocytes collected, all of

which were used for IVF, seven were fertilized but none were of adequate quality for a transfer. Two of the patients had no embryo transfer as no oocytes were collected, the remaining 1 patient had 19 oocytes collected, 14 were used for ICSI, but none fertilised. The patient that underwent GIFT had embryo transfer on D5.

6.7 Frozen embryos post embryo transfer

Forty of the 59 patients either chose not to freeze any embryos or did not have embryos of adequate quality for freezing post embryo transfer.

5.8 Pregnancy outcomes and rates

The biochemical pregnancy rate was 39% at day 14 post embryo transfer indicated by a serum βhCG of greater than 10IU/L. Twenty of the 59 patients had a clinical pregnancy at the seven week ultrasound yielding a clinical pregnancy rate of 34%. A clinical pregnancy was deemed such by the presence of at least one fetal heart at the seven week ultrasound. The singleton rate was 22%, 6.8% of the patients had at least two fetal hearts at the seven week ultrasound and 3.4% of the patients had three foetal hearts at 7 week ultrasound.

7. Discussion

7.1 Demographics and Pregnancy History

The mean age of patients in our study was 30.5 years. This classifies the majority of our patients into a young group for assisted reproduction and hence a group at high risk for developing OHSS. Comparing our mean age to that in other similar studies, the mean age in the study by Imbar et al was 30.0 years in the fresh embryo transfer group which is exactly what we found.[40] The mean age in the study by Manzanares et al was 33.9 years for the GnRH-agonist induction cycle which puts our patients at higher risk for developing OHSS.[31]

Fifty one out of 59 patients had primary infertility, showing that a high percentage of patients had not successfully carried a pregnancy to viability before.

7.2 Pre-Treatment Work Up

As this study was aimed at those patients at high risk for OHSS it was interesting to note that of the patients included in the study, 39% had polycystic-like ovaries in the left ovary, while 43% showed polycystic-like ovaries in the right ovary. Since our study was aimed at finding the best possible trigger for final oocyte maturation in the patient with a high risk of developing OHSS, the above finding showed that the patients included in this study did fulfil the criteria for the at-risk patient.

The AMH levels had a wide range with those patients with the highest antral follicle count also having the highest AMH. Women with an AMH <1.1ng/ml were excluded from the study. The lowest AMH included was 1.6ng/ml which was above the 1.1ng/ml limit chosen. The high level of AMH amongst the study population was also indicative that these patients were at high risk for developing OHSS.

7.3 Cycle-Specific Data

Of interest was the clinicians' preference to use Menopur® as the drug of choice for controlled ovarian stimulation. The biochemical pregnancy rate with Menopur® when used for stimulation was 38% as opposed to the biochemical pregnancy rate of 20% when Gonal-F® was used. When a combination of Gonal-F® and Menopur® were used, the biochemical pregnancy rate was 37%. The choice of which drug to use for stimulation was based purely on the clinician's opinion and the response of the patients involved. If a patient's response was poor then the drug was changed. Some patients were known to have had poor response from one of the drugs and were then stimulated with the other. There was no formula or specific protocol in place for choice of drug for stimulation and perhaps a randomised control trial may be needed to fully evaluate the best option for ovarian stimulation.

7.4 Appropriate use of GnRH-agonist Trigger

In the study by Aljawoan et al, quoted in the literature review, patients with more than 20 follicles at the time of ovulation induction were examined. Among this group of patients, 10.4% of those that had three leading follicles greater than 15mm and a serum estradiol level greater than 1635pg/ml (±6000pmol/L) developed moderate to severe OHSS[16]. In a review by Humaidan and Kol published in the Reproductive Biomedicine Online as recently as 2013, the most important primary risk factors for OHSS were a high AMH, high antral follicle count, PCOS or isolated PCOS characteristics and a previous history of OHSS. Their recommendation for when to trigger with a GnRH-agonist was when there are more than 14 follicles greater than 11mm on the day of trigger.[45] The results of our study showed that 48 out of 59 patients had more than 18 leading follicles with

the mean leading follicle size being 16.8mm. This puts the majority of patients in the study at high risk for developing OHSS, however only 1 patient developed moderate OHSS. The pre- trigger E2l levels with a median of 19 552pmol/L also indicate that these patients were at great risk for OHSS. Papanikolaou et al in Fertility and Sterility showed that a threshold of 18 or more leading follicles and or a pre-trigger estradiol level of greater than ±6000pmol/L yielded an 83% sensitivity and 84% specificity for severe cases of OHSS.[25]

Interestingly only seven patients in our study required coasting which implies that they were not hyper-stimulated.

7.5 Cycle Outcomes

Only 2 patients did not have oocytes collected and three patients had more than 20 oocytes with a mean number of 12 oocytes collected- this shows a COH with good outcomes with regard to oocytes collected. IVF was by far the most popular method of assisted reproduction. The fertilization rates of 93% showed good oocyte quality. Day 3 was the most popular day for embryo transfer. No patients underwent frozen embryo transfer on this study.

7.6 Pregnancy Outcomes

In the study by Manzanares et al, embryo transfer was conducted using only frozen embryos. The pregnancy rate quoted in this study is 33%.[31]

In the meta-analysis by Griesinger, clinical pregnancy rates were significantly reduced using a GnRH-agonist for ovulation induction with an odds ratio of 0.22, (95% CI = 0.05-0.85, p=0.03), and the study concluded that GnRH-agonist triggering significantly reduced the clinical pregnancy when fresh embryos were transferred in the treatment cycle.[33]

The Cochrane review by Youssef et al showed similar results to the meta-analysis by Griesinger et al and also recommended that GnRH-agonists should not be used for final oocyte maturation when fresh embryo transfer was taking place.[34]

In the prospective observational study by Griesinger et al quoted in the literature review, the on-going pregnancy rates in a “freeze all policy” when using a GnRH-agonist for final oocyte maturation was found to be 31.6%.[38]

Imbar et al however, showed in a 2012 study that the clinical pregnancy rate of fresh embryo transfer was 37% when the GnRH-agonist was used for final oocyte maturation.[40] In that study an intensive luteal phase support including 50mg of intra-muscular progesterone and 6mg of 17-β-estradiol was utilised similar to the luteal phase support used in our study. The pregnancy rates in our study were similar to those described in the study by Imbar et al and greater than the pregnancy outcomes in the meta-analysis by Griesinger quoted above.[34]

8. Limitations

One of the limitations of this study was that live birth rate was not represented. According to an opinion published in the Journal of Reproductive Genetics, live birth rate is the gold standard for the measurement of success in assisted reproduction.[46] The reason for not including live birth rate in this study is that patients were only followed up for a maximum of 10 weeks gestation. The Vitalab Centre for Assisted Conception does not have an obstetrics unit and once patients have been scanned at seven and ten weeks and are found to have normal on-going pregnancies they are then referred to either an obstetrician recommended by the clinic or to an obstetrician of the patient's choice. All patients under-going treatment at the Vitalab Centre for Assisted Conception have given consent for their records to be used for medical research, however these patients have not given permission to be contacted to find out about the outcome of the pregnancy. Although many of the patients or obstetricians that the patients consult will inform the clinic of the outcome of the pregnancy, this information was not available in the patient's file. For this reason live birth was not used as a variable in the success of the assisted reproduction although the researcher feels this would have added weight to the study.

A further limitation is that only one institution was used to collect data. If more than one clinic was used it would have added to the total number of patients, possibly excluded any bias with regard to patient selection and physicians possible preference for choice of drugs. As the luteal phase support protocol at the Vitalab Centre for Assisted Conception is also uniform across all the attending physicians, the possibility of the luteal phase support being the crucial factor in the success of the reproductive cycles was also not fully evaluated. Had the research taken place

at other centres, the luteal phase support would most likely have had some variety and this would have assisted the researcher in deciding how much of an impact the luteal phase support has had on the pregnancy outcomes.

A further limitation to this study is that the data collected were compared to similar studies in the literature but not compared to patients at our centre that were triggered with hCG. However as set out in the objectives of this study, the aim of this study was to assess pregnancy outcomes in a group of patients that were high risk of developing OHSS and hence were triggered with Lucrin. We also set out to show that transferring fresh embryos in a cycle where Lucrin was used as a trigger for ovulation as opposed to freezing the embyos does not impact on the pregnancy rates as compared with data published internationally.

The author does however acknowledge that a more suitable and scientifically sound approach would have been to perform a prospective study by randomising patients triggered with Lucrin into fresh embyo transfer group and a group that underwent frozen embryo transfer in a natural cycle.

Lastly, the stimulation protocols used in this study were not fixed for each patient but rather decided on by the clinician, which meant that the stimulation drugs used were not uniform throughout the study.

9. Conclusion

The mean age of 30.5 years, high antral follicle counts, high AMH levels, a large number of patients with more than 18 leading follicles with a mean size of 16.8mm at the time of the trigger and the high mean estradiol levels pre-trigger make this group high risk for developing OHSS.

Our study shows that the use of GnRH-agonists to trigger final oocyte maturation in assisted reproduction is associated with a low incidence of OHSS in high risk patients. Our findings are similar to other published data and support other such studies.

This study, to the best of the researcher's knowledge, is the first of its kind to be done in South Africa. We have shown the biochemical and clinical pregnancy rates to be acceptable when GnRH-agonists are used for final oocyte maturation and embryos are transferred fresh in the medicated cycle. This evidence suggests that embryos should not be frozen as this worsens the stress and time constraints of a couple undergoing assisted reproduction without an improvement in the outcomes.

The difference in pregnancy outcomes in patient stimulated with Menopur® versus Gonal-F® needs further work and may just be coincidental.

The intensive luteal phase support used by Vitalab Centre for Assisted Conception, may explain the excellent biochemical and clinical pregnancy rates. Further work needs to be done to ascertain whether the pregnancy rates would be as good with the use of less intense luteal phase support therapy.

10. References

1. Domar A, Gordon K, Garcia-Velasco J, La Marca A, Barriere P, Beligotti F. Understanding the perceptions of and emotional barriers to infertility treatment: a survey in four European countries. Hum Reprod 2012;27(4):1073-9.
2. Vitalab Centre For Assisted Conception. Available at http://www.vitalab.com/clinic/costing/ (Accessed on 2/9/2013)
3. Pinto-Gouveia J, Galhardo A, Cunha M, Matos M. Protective emotional regulation processes towards adjustment in infertile patients. Hum Fertil 2012;15(1):27-34.
4. Steptoe PC, Edwards RG. Birth after the re-implantation of a human embryo. Lancet 1978;12(2):366.
5. Trounson A, Leeton J, Besanko M, Wood C, Conti A. Pregnancy established in an infertile patient after transfer of a donated embryo fertilised in vitro. Br Med J 1983;12(286):835-8.
6. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 1992;340(8810):17-8.
7. Jouannet P. Evolution of assisted reproductive technologies. Bull. Acad. Natl. Med. 2009;193(3):573-82.
8. Odendaal HJ, Schaetzing AE, Kruger TF. Clinical Gynaecology 2nd Edition. Landsdowne: Juta Publishers; 2001.
9. Dunitz M. Textbook of Assisted Reproductive Techniques – Laboratory and Clinical Perspectives.London: Martin Dunitz Ltd. The Livery House; 2001.

10. Kyrou D, Kolibianakis EM, Fetami HM, Tarlatzis BC, Tournaye H, Devroey P. Is earlier administration of human chorionic gonadotropin associated with the probability of pregnancy in cycles stimulated with recombinant follicle-stimulating hormone and gonadotropin-releasing hormone antagonists? A prospective randomized trial. Fertil and Steril 2011;96(5):1112-5.
11. Kolibianakis EM, Griesinger G, Venetis CA. GnRH-agonist for triggering final oocyte maturation: time for a critical evaluation of data. Hum Reprod Update. 2012;18(2):228-9.
12. Royal College of Nursing. “Performing Ultrasound Guided Oocyte Retrieval.” 2004. Available from: http://www.rcn.org.uk/__data/assets/pdf_file/0004/78619/002425.pdf [Accessed on 22 Jan 2013].
13. Urman RD, Gross WL, Phillip BK. Anaesthesia outside of the operating room. New York: Oxford University Press, 2011.
14. Brinsden PR ed. A textbook of In Vitro Fertilization and Assisted Reproduction 2nd edition. New York: The Panthenon Publishing Group Inc, 1999.
15. Swain JE, Smith GD. Advances in embryo culture platforms: novel approaches to improve pre-implantation embryo development through modifications of the microenvironment. Hum Reprod Update. 2011;17(4):541-57.
16. Aljawoan FY, Hunt LP, Gordon UD. Prediction of ovarian hyperstimulation syndrome in coasted patients in an IVF/ICSI program. J Human Reprod Sci. 2012;5(1):32-36.

17. Papanikolaou EG, Humaidan P, Polyzos NP, Tarlatzis B: Identification of the high-risk patient for ovarian hyperstimulation syndrome: Semin. Reprod Med. 2010;28(6):458-462.
18. Mansour R, Aboulghar M, Serour G, Amin Y, Abou-Setta AM .Criteria of a successful coasting protocol for the prevention of severe ovarian hyperstimulation syndrome. Hum Reprod. 2005;20(11):3163-3172.
19. Royal College of Obstetricians and Gynaecologists. The Management of Ovarian Hyperstimulation Syndrome.Green-top Guidline No.5. London RCOG press, 2006
20. Delvigne A, Demoulin A, Smitz J, Donnez J, Koninckx P, Dhont M, et al. The ovarian hyperstimulation syndrome in in-vitro fertilization: a Belgian multicentric study. I. Clinical and biological features. Hum Reprod. 1993;8(9):1353–60.
21. Golan A, Weissman A. A modern classification of OHSS. Reprod Biomed Online. 2009;19(1)
22. Binder H, Dittrich R, Einhaus F, Krieg J, Müller A, Strauss R, et al. Update on ovarian hyperstimulation syndrome: Part 1—incidence and pathogenesis. Int J Fertil Womens Med. 2007;52(1):11–26.
23. Mathur RS, Akande AV, Keay SD, Hunt LP, Jenkins JM. Distinction between early and late ovarian hyperstimulation. Fert and Sterl. 2000;73(5):901-7.
24. Humaidan P, Quatrarolo J, Papanikolaou EG. Preventing ovarian hyperstimulatiom syndrome: guidance for the clinician. Fertiland Steril. 2010;94(2):389-400.

25. Papanikalaou EG, Humaidan P, Polyzos NP, Tarlatzis B: Identification of the high risk patient for ovarian hyperstimulation syndrome. Semin Reprod Med. 2010;28(6):458-462.
26. Fiedler K, Ezcurra D: Predicting and preventing ovarian hyperstimulation syndrome (OHSS): the need for individualised not standardized treatment. Reprod Biol and Endocrinol. 2012;24:10-32.
27. Levinsohn-Tavor O, Friedler S, Schachter M, Raziel A, Strassburger D, Ron-El R. Coasting – what is the best formula? Hum Reprod. 2003;18(5):937-940.
28. Humaidan P, Kol S, Papanikolaou EG. GnRH-agonist for triggering of final oocyte maturation: time for a change of practice? Hum Reprod Update 2011;17(4):510-524.
29. Itskovitz-Eldor J, Kol S, Mannaerts B: Use of a single bolus of GnRH-agonist triptorelin to trigger ovulation after GnRH antagonist ganirelix treatment in women undergoing ovarian stimulation for assisted reproduction, with special reference to the prevention of ovarian hyperstimulation syndrome: preliminary report: Short communication. Hum Reprod. 2000;15(9):1965-8.
30. Al-Inany HG, Youssef MA, Aboulghar M, Broekmans F, Sterrenburg M, Smit J, Abou-Setta AM. GnRH-antagonists are safer than agonists: an update of a Cochrane review. Human Reproduction Update. 2011;17(4):435.
31. Manzanares MA, Gomez-Palomares JL, Ricciarelli E, Hernandez ER. Triggering ovulation with gonadotropin-releasing hormone agonist in in vitro fertilization patients with polycystic ovaries does not cause ovarian

hyperstimulation sundrome despite very high estradiol levels. Fert and Steril. 2010;93(4):1215-19.

32. Copperman AB, Benadiva C. Optimal usage of the GnRH-antagonists: a review of the literature. Reprod Biol Endocrinol. 2013;15(11):20
33. Griesinger G, Diedrich K, Devroey P, Kolibianakis EM. GnRH-agonist triggering final oocyte maturation in the GnRH antagonist ovarian hyperstimulation protocol: a systematic review and meta-analysis. Hum Reprod Update. 2006;12(2):159-168
34. Youssef MA, Van der Veen F, Al-Inany HG, Griesinger G, Mochtar MH, van Wely M. Gonadotropin-releasing hormone agonist versus hCG for oocyte triggering in antagonist assisted reproductive technology cycles. Cochrane Database Syst Rev. 2010;10:(11).
35. Griesinger G, von Otte S, Schroer A, Ludwig AK, Diedrich K, Al-Hasani S, Schultze-Mosgau A. Elective cryopreservation of all pronuclear oocytes after GnRH-agonist triggering of final oocyte maturation in patients at risk of developing OHSS: a prospective observational proof-of-concept study. Hum Reprod. 2007;22:(5):1348-1352.
36. Ghobara T, Vandekerckhove P. Cycle regimens for frozen-thawed embryo transfer. Cochrane Database Syst Rev. 2008;23:(1).
37. Devroey P, Polyzos N.P.,Blockeel C. An OHSS free clinic by segmentation of IVF treatment. Hum Reprod. 2011;26(10):2593-7.
38. Griesinger G., Schultz L., Bauer T., Broessner A., Frambach T., Kissler S. Ovarian hyperstimulation syndrome prevention by GnRH afonst triggering of final oocyte maturation in a GnRH antagonist protocol in combination

with a “freeze all” strategy: a prospective multicentric study. Fertil. Steril. 2011;95:2029-2033.

39. Benadiva C, Engmann L. Intensive luteal phase support after GnRH-agonist trigger: does it help? Reprod Biomed Online. 2012;25:329-330.
40. Imbar T, Kol S, Lossos F, Bdolah Y ,Hurwitz A, Haimov-Kochman R. Reproductive outcome of fresh or frozen –thawed embryo transfer is similar in high-risk patients for ovarian hyperstimulation syndrome using GnRH-agonist for final oocyte maturation and intensive luteal phase support. Hum. Reprod. 2012;27:753-759.
41. van der Linden M, Buckingham K, Farquhar C, Kremer JA, Metwally M. Luteal phase support for assisted reproduction cycles. Cochrane Database Syst Rev. 2011;5:(10)
42. Van Rooij IA, Broekmans FJ, Scheffer GJ, Looman CW, Habbema JD, de Jong FH. Serum AMH levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fert and Sterl. 2005;83(4):979-87.
43. Klinkert ER, Broekmans FJM, Looman CWN, Habbema JDF, te Velde ER. The antral follicle count is a better marker than basal follicle stimulating hormone for the selection of older patients with acceptable pregnancy prospects after in vitro fertilization. Fert and Sterl. 2005;83(3):811-4.
44. Toth A, Lesser M. Outcome Of Subsequent IVF Cycles Following Antibiotic Therapy After Primary Or Multiple Previously Failed ICVF Cycles. The Internet Journal of Gynecology and Obstetrics. 2007;7(1). < http://archive.ispub.com/journal/the-internet-journal-of-gynecology-and-obstetrics/volume-7-number-1/outcome-of-subsequent-ivf-cycles-following-

antibiotic-therapy-after-primary-or-multiple-previously-failed-ivf-cycles.html#sthash.DZT40vhq.dpuf> Last seen 2/9/2013.

45. Kol S, Humaidan P. GnRH-agonist triggering: recent developments. Reprod Biomed Online. 2013;26(3):226-30.

46. Ulug U, Ben-Shlomo I, Bahceci M. Opinion: How should we define success in assisted reproduction? Is live birth rate the gold standard measurement? J Assist Reprod Genet. 2010;27(12):691-3.

APPENDIX A: DATA SHEET

Past History

Patient # - _____ Age - _____

Parity - _____Gravidity - _____

Miscarriages - _____Ectopics - _____

Pre – treatment AFC (Antral follicle count) -RIGHT _____ LEFT_____

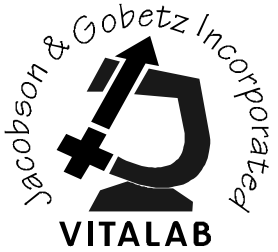
Pre – treatment AMH (Anti-Mullerian hormone) - _____

Pre-treatment FSH (Follicle stimulating hormone)_____

Cycle data

Day 2 Estrogen	
Day 2 Progesterone	
Stimulation drug used	
Stimulation duration	
Stimulation dose used	
Number of leading follicles	<18 >18
Size of leading follicles	
Estrogen value pre-trigger	
Progesterone pre-trigger	
Trigger drug	
Trigger dose	
Number of ocytes collected	
# of oocytes for IVF	
# of oocytes for ICSI	
# of oocytes for ZIFT	
# of oocytes for GIFT	
Total fertilised	
Day transferred	
# of frozen embryos	
B-hCG – 1	
B-hCG – 2	
B-hCG – 3	
Sonar - # of gestational sac	
Sonar - # of fetal hearts	

APPENDIX B: Consent form signed by patients



CONSENT FORM FOR PARTICIPATION IN THE

ASSISTED REPRODUCTION PROGRAMME INCLUDING :

A) IN-VITRO FERTILISATION AND EMBRYO TRANSFER - IVF

B) INTRACYTOPLASMIC SPERM INJECTION - ICSI

C) GAMETE INTRA-FALLOPIAN TRANSFER - GIFT

D) OTHER ASSISTED REPRODUCTIVE TECHNOLOGIES

1) We hereby authorise and direct the Gynaecologist(s), Drs Jacobson, Gobetz and Volschenk and such assistants as may be selected by them to administer and to treat

_____

FULL NAMES OF COUPLE

In accordance with the protocols which we have read and which have been discussed with us, and we hereby consent to such treatment.

2) We understand that it may be necessary to produce a semen (sperm) sample to be frozen. This will be used for back-up purposes and will be thawed on the day of oocyte retrieval. If it is not required, it will be discarded after the procedure.

3) We understand that medical aid coverage for any or all of the above procedures may not be available and that we are personally responsible for the expenses incurred during treatment. These expenses have been discussed with me by the medical team.

4) We understand that we are free to discontinue participation in the treatment programme at any time, either verbally or in writing. We also understand that, if we do decide to discontinue participation in the programme, we will be responsible for all expenses incurred during the period prior to discontinuation and which relate to such treatment.

5) We understand that this consent extends from the original period in the treatment programme until the treatment is completed or until we decide to discontinue participation. Further, this consent is binding for participation in subsequent treatment programme.

6) We understand that, should the results of my treatment or any aspect of it be published in medical or scientific journals, all possible precautions will be taken to protect my anonymity. We grant permission to the medical team to publish in professional journals relating to our case, provided our names are not used.

7) We have read and understand this consent form and all our questions have been answered to our satisfaction. All blanks were filled prior to signature.

DATE: ……..

SIGNATURE OF WOMAN: ……

SIGNATURE OF MAN: ……

SIGNATURE OF DOCTOR: ……

SIGNATURE OF PERSON OBTAINING CONSENT: …….

WITNESS: ……..

APPENDIX C: ETHICS CLEARANCE

M130241M130241

R14/49 Dr Yosef Y Unterslak

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M130241

NAME: (Principal Investigator)	Dr Yosef Y Unterslak
DEPARTMENT:	Department of Obstetrics & Gynaecology CM Johannesburg Academic Hospital
PROJECT TITLE:	A Descriptive Study on the Use of Gonadotropin Releasing Hormone Agonist for Assisted Reproductive Tecnhiques (revised title)
DATE CONSIDERED:	22/02/2013
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr T Hassim
APPROVED BY:	Professor PE Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 23/08/2013

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature M130241Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES