THE IMPACT OF GnRH-AGONIST TRIGGERS ON AUTOLOGOUS IN VITRO FERTILIZATION OUTCOMES: A RETROSPECTIVE ANALYSIS

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2019

DECLARATION

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LIST OF ABBREVIATIONS:

AFC	**antral follicle count**
AMH	**anti-mullerian hormone**
CL	**corpus luteum**
COS	**controlled ovarian stimulation**
ET	**embryo transfer**
FET	**frozen embryo transfer**
FSH	**follicle stimulating hormone**
GnRH	**gonadotropin releasing hormone**
GnRHa	**gonadotropin releasing hormone agonist**
hCG	**human chorionic gonadotropin**
ICSI	**intracytoplasmic sperm injection**
IVF	**in vitro fertilization**
LH	**luteinising hormone**
OHSS	**ovarian hyperstimulation syndrome**
OA	**oocyte aspiration**
OS	**ovarian stimulation**

1. INTRODUCTION & BACKGROUND

In vitro fertilization in assisted reproduction requires ovarian stimulation (OS) with exogenous gonadotrophins and oocyte maturation before ultrasound guided aspiration. With the evolution of controlled ovarian stimulation, two specific oocyte maturation triggers have been utilized, mainly human chorionic gonadotrophin (hCG) and GnRH-agonists (*Humaidan et al., 2011*). While hCG allows for easier luteal phase support, GnRH-agonists have been associated with lower incidence of ovarian hyperstimulation syndrome *(Kol et al., 2004)*. As many fertility patients are either young donors or otherwise healthy patients, every effort has been made to reduce the incidence of ovarian hyperstimulation syndrome (Humaidan et al., 2015).

Ovarian hyperstimulation syndrome (OHSS) is one of the most severe complications of ovarian stimulation in IVF cycles. Hyperstimulation results in the shifting of fluid from the intravascular space due to an increase in vascular permeability (*Busso et al., 2017).* OHSS can be life-threatening with consequences such as renal failure, thrombosis, and third space fluid shifts (ascites, pleural effusions) (*Busso et al., 2017)*. All of these serious complications can occur in otherwise young and healthy patients. The reduction in risk of OHSS with GnRH-agonist trigger for oocyte-maturation is well documented *(Kol et al., 2004; Itskovitz-Eldor et al., 2000; Engmann et al., 2008; DiLuigi et al., 2010)*.

In order for a transferred embryo to implant, the endometrium must be in synchrony with ovarian events and receptive to be able to support implantation. In a natural menstrual cycle, the endometrium is influenced by specific hormonal events. These include oestrogen stimulated proliferation and progesterone receptor induction. Upon ovulation, the resultant corpus luteum secretes progesterone. This transforms a proliferative endometrium to a secretory one. For the first seven weeks of pregnancy, the corpus luteum (CL) maintains progesterone secretion (*Mitwally et al., 2010*). However, after nine weeks of gestation, the syncytiotrophoblast maintains progesterone production, with the interim period supported by both the CL and syncytiotrophoblast (*Mitwally et al., 2010*).

The luteal phase in IVF cycles always requires supplementation. Controlled ovarian stimulation (COS) usually results in supra-physiological levels of oestrogen which can precipitate an endogenous luteinizing hormone (LH) surge and subsequent ovulation. Therefore, suppression of endogenous LH is essential but this effect may disrupt corpus luteal function, resulting in inadequate progesterone secretion despite the presence of multiple corpora lutea. A further contributing factor is the disruption of granulosa cells at the time of oocyte retrieval *(Humaidan et al., 2011)*.

The amount of progesterone support needed in the luteal phase varies according to the selected type of oocyte maturation trigger. When hCG is used for oocyte maturation, it acts more potently than natural LH, and has a longer duration of action (*Hoff et al*., 1983; *Weissman et al., 1996)*. The injection of 10 000 units of hCG may be sufficient to entirely rescue the luteal phase, but some studies have demonstrated a higher pregnancy rate with a small dose of additional progesterone supplementation in hCG triggered IVF cycles *(Lui et al., 2012)*. Doses of progesterone in this review ranged from 200mg vaginal progesterone given two or three times per day, to 50mg of intramuscular progesterone up to 4 times per day.

The GnRH-agonist trigger causes a direct down-regulation of the pituitary and does not support the corpora lutea with sufficient LH stimulation after its immediate action over 24-48 hours (*Humaidan et al., 2011)*. This results in decreased corpus luteum activity, subsequent lower progesterone levels, and a sub-optimum endometrium for implantation. Significantly lower pregnancy rates have been demonstrated when progesterone supplementation is omitted in these cycles, and most patients menstruate within 7-10 days if full luteal support is not provided (*Humaidan et al., 2011*).

Progesterone support can be administered via vaginal, intramuscular or subcutaneous preparations (*Ganesh et al., 2011)*. There is no clear global consensus on the optimal progesterone supplementation protocol (*Humaidan et al., 2015*). Vaginal preparations, in doses ranging from 90mg daily to 200mg three times daily (depending on the exact preparation), which rely on the local effect of progesterone on the endometrium, have a higher patient acceptance and lower side effect profile (*Levine, 2000*). Intramuscular progesterone allows reliable systemic levels of progesterone but results in discomfort

at the injection site, including cold abscess formation in some cases (*Lockwood et al., 2013*). Additionally, allergic reactions can occur due to the delivery systems used in the depot formulations. Subcutaneous progesterone may be a new good alternative but is not universally available and has a significant cost factor.

The aim of this study was to determine if differences exist in pregnancy rates between supplemented GnRH-agonist triggers (supported with high dose luteal phase progesterone (100mg intramuscular Gestone) and oral oestrogen) and hCG triggers for oocyte maturation.

2. LITERATURE REVIEW

2.1.1 GnRH-Agonist Trigger Reduces OHSS

Ovarian hyperstimulation syndrome is one of the most severe complications of COS with a varying incidence depending on severity. OHSS is diagnosed clinically and graded as mild, moderate, severe, or critical *(Royal College of Obstetricians & Gynaecologists, 2016)*. American data showed an incidence of 1.1%, while the 14th European IVF-Monitoring report showed a lower hospitalisation rate of 0.3% in 2010 *(Royal College of Obstetricians & Gynaecologists, 2016)*. With the trend in delayed childbearing, there is an increased need for oocyte donation (*Johnson et al., 2012*). Oocyte donors are often young, and are at significant risk for OHSS and hospitalization. Other groups at risk include patients with polycystic ovarian syndrome, those with high indicators of ovarian reserve i.e. high Anti-Mullerian Hormone levels (AMH) or high Antral Follicle Counts (AFC), and patients with a prior history of OHSS *(Royal College of Obstetricians & Gynaecologists, 2016; Busso et al., 2017)*.

The hyperstimulated ovary exposed to human chorionic gonadotrophin (hCG) leads to proinflammatory mediator production, such as vascular endothelial growth factor (VEGF) and other cytokines, which in turn leads to increased vascular permeability and a pro-thrombotic effect (*Royal College of Obstetricians & Gynaecologists,* 2016; *Busso et al., 2017*). While a variety of methods have been employed to prevent OHSS, the most effective has been the use of GNRH-agonists to trigger final oocyte maturation, in place of hCG administration (*Humaidan et al., 2015; Benadiva et al., 2018*). Other methods for prevention of OHSS include cycle cancellation, withholding hCG trigger, withdrawing exogenous gonadotrophins and withholding hCG in patients on a GnRH-agonist long protocol cycle, cryopreservation of all embryos and transfer in a subsequent cycle, intravenous albumin administration; and use of dopamine agonists (*Aboulghar, 2009*).

Interest in GnRH agonist-triggers surged in the 1980's and early 1990's when the use of this drug was shown to induce oocyte maturation and ovulation *(Gonen et al., 1990)*.

However, this interest was limited by the use of GnRH-agonist for pituitary down-regulation at the time.

With the introduction of the GnRH-antagonist IVF protocol to prevent the premature LH surge in IVF cycles, it became apparent that adding GnRH-agonists at the time of full follicular development would displace GnRH-antagonists at the level of the pituitary. The GnRH receptor does not undergo down-regulation when bound by a GnRH-antagonist. When used as an oocyte maturation trigger, the GnRH-agonist competitively displaces the GnRH-antagonist from the receptor. This results in activation of the receptor, release of gonadotrophins, and induction of ovulation (*Humaidan et al., 2011*).

Important differences exist when comparing the gonadotrophin surge that occurs in a natural cycle and the GnRH-agonist induced surge. Of importance is the shorter duration of LH surge, which lasts between 24 and 36 hours *(Itskovitz et al., 1991).* This is significantly shorter in comparison to the natural cycle LH surge of 48 hours (*Hoff et al., 1983)*. The shorter duration of gonadotrophin release in GnRH-agonist surges may result in a defective luteal phase and resultant need for modified luteal phase support (*Humaidan et al., 2011*). In addition to the shorter gonadotrophin release, supra-physiological levels of ovarian steroids during stimulation may also result in negative-feedback at the hypothalamic-pituitary-axis, and further suppression of LH secretion (*Fatemi, 2009*). The significance of LH is both in its crucial role in supporting progesterone production by the corpus luteum, along with up-regulation of growth factors involved in implantation (*Humaidan et al., 2011*) and effect on endometrial LH receptor expression (*Rao et al., 2001)*. Many studies show a lower pregnancy rate for GnRH-agonist triggered IVF cycles when standard low dose progesterone supplementation is utilized, such as those used for hCG-triggered IVF cycles, or when no luteal support is provided (*Humaidan et al., 2005; Kolibianakis et al., 2005*; *Griesinger et al.,2006; Youssef et al., 2014)*.

2.1.2 Initial GnRH-Agonist Trigger Studies

When reviewing the literature on GnRH-agonists, it is important to understand the evolution of their use in IVF cycles and the specific luteal phase support used in each cycle. The literature discussed below has been specifically chosen to demonstrate the

scientific evolution of our understanding of the GnRH-agonist trigger, and highlight the origin of the concerns that still linger today.

The initial studies involving GnRH-agonists for oocyte maturation raised concerns about lower pregnancy and higher miscarriage rates. An early study by *Itskovitz et al (2000)* raised similar concerns about GnRH-agonist triggering for oocyte maturation. Eight patients considered to be at risk of OHSS (by number of follicles and oestrogen levels on day of trigger) were given GnRH-agonists instead of hCG for final oocyte maturation. The luteal phase was supported with 50mg of intramuscular progesterone (Gestone) and 2mg of oral oestrogen. Only one out of seven fresh transfers resulted in a pregnancy while 4 out of 17 of frozen-thawed cycles resulted in a pregnancy, with three of these ending in early losses. The authors do not detail how the patient's endometria were prepared for frozen embryo transfers. They concluded that the effect of GnRH-agonists was not only limited to the endometrium alone, but possibly impacted on the developmental capacity of the embryos themselves. This was later disproven in larger frozen-thawed cycles, but raised initial concerns about increased miscarriage rates with GnRH-agonist triggers (*Griesinger et al., 2007*). The luteal phase support in the Itskovitz study was suboptimal by current progesterone dosing standards (100mg Gestone), and this may explain the low success rates.

A retrospective cohort study by *Bracero et al (2001)* compared implantation rates in 8 and 11 patients triggered with GnRH-agonists and hCG respectively for final oocyte maturation. The luteal phase was solely supported with six-hourly micronised vaginal progesterone. Both arms had comparatively low implantation rates.

A prospective randomised three-arm study was published in 2002 by *Fauser et al.* In this study, two GnRH-agonists (Triptorelin and Leuprorelin) were compared to hCG as oocyte maturation triggers in 47 intracytoplasmic sperm injection (ICSI) cycles. The luteal phase was only supported with 50mg of intramuscular progesterone in all study arms. Implantation and pregnancy rates were low in all three arms, but five out of the eleven (45.5%) of patients in the GnRH-agonist arm suffered early pregnancy losses, again likely due to low luteal phase progesterone dosing. These findings intensified concerns about the possible association between GnRH-agonist triggers and high early pregnancy loss rates.

One of the more significant studies assessing differences in pregnancy outcomes between GnRH-agonist and hCG triggers for oocyte maturation was published in 2005 *(Humaidan et al., 2005)*. This study randomised 122 normogonadotrophic women between the ages of 25 and 40 years to receive either hCG or GnRH-agonists as a trigger for oocyte maturation. The women were stimulated with standard doses of recombinant FSH (150u or 200u); GnRH antagonists were started when a follicle satisfied the 15mm size criterion, and triggering was commenced when three or more follicles measured 17mm in diameter on ultrasound. Oocyte retrieval was performed at 35 hours after trigger, and IVF or ICSI followed. A maximum of two embryos were transferred on day 2 or 3. The luteal phase was supported with daily doses of 90mg vaginal progesterone gel (8% Crinone) and 4mg of oral oestrogen (Estrofem) in all trigger arms. This was continued until the day of the pregnancy test.

Embryo transfer was cancelled in 7/55 patients in the GnRH-agonist and 10/67 of the patients in the hCG group due to the absence of fertilization or poor embryo quality. The groups were comparable by mean age, BMI, baseline FSH levels, total drug administered, IVF vs ICSI, and day 2 or 3 transfers. Importantly, no cases of OHSS were reported in either group. The results suggested that while GnRH-agonists were associated with a higher rate of MII (mature) oocytes (84% vs 68%, $p < 0.02$ log transformed), fertilization and cleavage rates were similar. These findings suggest that the advantage of a higher number of mature oocytes did not translate to improvement in fertilization or cleavage rates. The clinical pregnancy rates however were dramatically lower (6 vs 36%) and pregnancy losses higher (79% vs 4%) for patients in the GnRH-agonist in comparison to hCG triggers for oocyte maturation. The study was originally designed to have 150 patients in each arm but was prematurely discontinued at 122 participants due to concerns over very low pregnancy outcomes in the GnRH trigger group.

At the time of the findings report from the Humaidan study, another study (*Kolibianakis et al., 2005*) was underway to assess ongoing pregnancy rates with GnRH-agonist triggers. This study included 106 patients comparing GnRH-agonist to hCG triggers. The luteal phase was supported with vaginal progesterone (Uterogestan 600mg in three divided doses) and oral oestradiol 2mg twice daily in both trigger arms. Luteal

phase support was continued until 7 weeks of gestation in the case of positive pregnancy results. Pregnancies progressing after the 12th week of gestation were considered ongoing. Important findings from this study included amongst others, a similar number of mature oocytes, fertilization rates, and embryo quality between the two trigger types. Again, and in congruence with the *Humaidan* study, a significantly lower ongoing pregnancy rate was reported in the GnRH-agonist trigger group (OR 0.11, 95% CI 0.02 to 0.52). Additionally, no cases of ovarian hyperstimulation syndrome were described in this study.

The initial studies outlined above show conflicting results in terms of number of mature oocytes, fertilization rates, positive pregnancy outcomes, but altogether seemed to raise concerns about increased miscarriage rates.

2.1.3 Donor cycles

In order to address whether the low pregnancy and high miscarriage rates were due to the GnRH-agonist trigger itself, a Spanish study examined donor-recipient cycles in which half of the oocyte donors were triggered with GnRH-agonist and the other half with hCG (*Acevedo et al., 2006*). The study sample included 60 donors, stimulated with the same step-down GnRH-antagonist cycle, and then randomised to receive either a hCG or GnRH-agonist trigger for oocyte maturation. Endometrial preparation in the recipients was accomplished with oral oestrogen (no dose directive was given – the lining had to measure a minimum of 8mm on ultrasound), and this was followed with the administration of 600mg per day of micronised vaginal progesterone. The pregnancies were documented per transfer and were similar, with 55% in the GnRH-agonist trigger group and 59% in the hCG group ($p > 0.05$). Fourteen biochemical pregnancies were reported, of which 5 were in the GnRH-agonist group. Of the pregnancies documented, 84% were confirmed on scan in the GnRH-agonists group, compared to 90% in the hCG group. None of these results were of statistical significance.

A retrospective study including donor-recipient cycles between 2004 and 2015 also published similar results (*Irani et al., 2016*). In this study a total sample size of 1227 patients with only single triggers (i.e. no combination of GnRH-agonists and hCG) were included. Of these, 155 were in the GnRH-agonist trigger group, and the rest,

1072 were in the hCG group. Both groups had the same livebirth and implantation rates of 55% and 48% respectively. Taken together, these studies suggested that luteal phase support in GnRH-agonist triggered cycles warranted further investigation.

2.1.4 Results in Frozen-Thawed Cycles

Data from patients enrolled in the *Kolibianakis* and *Humaidan* studies (mentioned above) were further prospectively assessed in frozen-thawed cycles (*Griesinger et al., 2007*). These data were analysed in order to determine whether the poor pregnancy outcomes observed with GnRH trigger use were secondary to an effect on oocyte quality, embryo developmental capacity or luteal phase deficiency. In these two studies embryos were frozen on day 2 or 3 and were morphologically comparable in the hCG and GnRH-agonists groups. There was a similar thaw-survival rate in both groups. The endometrial lining was optimised with either a combination of oestrogen and progesterone, clomiphene citrate, or accomplished naturally. The livebirth rates per transfer in frozen-thawed cycles was 18% (95% CI, 8.2 to 36.7) and 30% (95% CI, 14.5 to 51.9) in the hCG and GnRH-agonist trigger groups respectively. The live-birth rate per patient starting a frozen-thawed cycle was 16% (95% CI, 7.1 to 32.6) and 26% (95% CI, 12.5 to 46.5) for hCG and GnRH-agonist triggers respectively. The study made no comment on miscarriage rates. The authors then concluded that lower pregnancy rates with GnRH trigger use were unlikely caused by a defect in the implantation potential of the embryos, but more likely by a deficiency in the luteal phase. This then heralded the search for the ideal luteal phase support regime.

2.2. The Luteal Phase: How Best to Support?

2.2.1 The European Approach

Some initial work on supplementation of the luteal phase in insemination cycles where a small hCG bolus was administered at 12 hours after GnRH-agonist trigger, had yielded encouraging results and this finding was further explored in a pilot study in 2006 (*Humaidan et al., 2006*). The pilot study population consisted of forty-five patients in three arms: the first arm received the hCG trigger; the second arm received the GnRH-agonist trigger and then an hCG bolus of 1500u 12 hours thereafter; and the third group's hCG bolus was delayed until 35 hours. Their stimulation was the same across all three groups, and the luteal phase was supported with oestrogen 4mg orally and vaginal progesterone (90mg 8% Crinone) daily. Although poor embryo

development was seen in all three groups, there was a notably lower pregnancy rate in the third (35 hour hCG) group. This group also had the lowest mid-luteal progesterone levels, suggesting poorer corpus luteul support. The study reported no pregnancy losses nor cases of OHSS (however at-risk patients were excluded).

The findings of the above study were confirmed in a larger randomized study of 305 cycles comparing hCG trigger with GnRH-agonist trigger and hCG bolus at 35 hours (*Humaidan et al., 2010*). The relative risk for early pregnancy loss in the GnRH-agonist plus hCG bolus group was 1.3 (21% in the GnRH-agonist plus hCG group, and 17% in the hCG alone group) but this was not statistically significant. The positive pregnancy rate was equivocal at 48% (OR 1.0, 95% CI 0.9 to 1.2), and the ongoing pregnancy rate and delivery rates were both 0.7 (95% CI 0.6-0.8 for both). Importantly, as the patients were randomised and not chosen for either trigger, the hCG trigger group had three cases of OHSS. The GnRH-agonist group (with additional hCG) had none, suggesting higher risk of OHSS with hCG triggers in comparison to GnRH-agonists (even with the additional hCG bolus).

With the new promise of luteal phase salvation with hCG, the focus was then on investigating the optimal dose of hCG. A non-randomised study of 192 patients deemed at risk of OHSS due to excessive response to controlled ovarian stimulation was conducted in which all patients were triggered with GnRH-agonists and hCG boluses as detailed below (*Castillo et al., 2010*). All patients were given 600mg of vaginal progesterone in addition to the hCG boluses for luteal support. The boluses were administered every third day as follows: OA +1 day, OA + 4 days, and OA +7 days (OA referring to day of oocyte aspiration). The three different doses were 1000 IU hCG (44 patients), 500 IU hCG (115 patients), and 250 IU hCG (33 patients). The pregnancy rates in each group were 47.7%, 42.6%, and 39.4% respectively (differences in pregnancy rates were not statistically significant). Ovarian hyperstimulation syndrome was seen in 15 out of the 192 patients, with the majority being late onset, including 4 in twin pregnancies. Six out of fifteen (40%) patients with OHSS were in the 1000 IU hCG bolus group. The authors concluded that although there was evidence to avoid the higher dose, hCG still demonstrated sufficient rescue of the luteal phase. The incidence of OHSS is still significant, and raises the question about hCG supplementation in any form in high risk patients. Furthermore, the authors

argued for single embryo transfers in all high responder patients, although there is no evidence that this will ultimately reduce OHSS once pregnancy occurs.

The question of OHSS risk stratification and personalized luteal phase support was partially addressed in an incomplete study in 2013 (*Humaidan et al., 2013)*. A cohort of 390 women with an antral follicle count of less than 25 were enrolled into the study. The patients were all stimulated and on the day of ovulation induction, the number of follicles above 11mm were noted on transvaginal ultrasound. Those with follicle number above 14 were placed into a “high risk group” and randomised to either 5000 IU hCG or GnRH-agonist plus 1500 IU hCG at 35 hours post-trigger. The hCG alone group had two cases of moderate late-onset OHSS, while the GnRH-agonist plus hCG group had none. There were no significant differences in pregnancy rates, ongoing pregnancy rates, or early pregnancy losses between the two trigger groups. The second patient group of less than 14 follicles of 11mm in size were similarly randomised to hCG 5000 IU on day of trigger, or GnRH-agonist plus 1500 IU hCG at 35 hours, with additional hCG (1500 IU) at 5 days post-retrieval. Interestingly, the latter group had two cases of severe late-onset OHSS, versus none in the hCG alone group. These OHSS patients had 11 and 13 oocytes retrieved and the authors argued they were very close to being in the “high risk” group. Again, there were no significant differences in pregnancy rates, ongoing pregnancy rates, or early pregnancy losses. This study, unfortunately not completed (due to an author’s death), suggested that hCG bolus supplementation to GnRH trigger could be safe in patients deemed at risk of OHSS.

2.2.2 Recombinant LH Luteal Supplementation

Another group directly investigated the possibility of LH-deficiency with GnRH trigger in a randomised controlled trial. In this RCT, patients who received the GnRH-agonist trigger were supplemented with additional LH (*Papanikolaou et al., 2011*). Thirty-five participants were stimulated with 187.5 IU of recombinant FSH and then randomised to either hCG with 600mg of micronised vaginal progesterone as luteal support, or GnRH-agonist trigger with 600mg of micronised vaginal progesterone plus a 300 IU dose of recombinant LH on the day of retrieval and every second day thereafter until day 10 post-retrieval. The baseline characteristics of the study cohort were similar, including stimulation length, number of oocytes retrieved and fertilization rates. Two

patients in each group did not have a transfer due to embryos failing to reach the blastocyst stage. The implantation rates for the hCG group were 26.7% versus 31.2% in the LH group ($p < 0.91$). There were no cases of OHSS, but the authors commented that the patients in the study were not high risk for OHSS and therefore inferences for OHSS could not be made.

2.2.3 The Exogenous Progesterone-Free Luteal Support

Another novel strategy for luteal support included daily hCG boluses from the time of trigger to the time of pregnancy testing (*Andersen et al., 2016).* This was based on the original pilot study of 15 normo-responders with a prior failed IVF cycle after hCG trigger (*Kol et al., 2011*). The patients all demonstrated between 5 and 12 follicles in the cycle. The hCG bolus was administered on the day of oocyte retrieval (1500 IU hCG), and again four days later. The ongoing pregnancy rate was 47% in this small group. As the patients were not high-risk for OHSS, it was not surprising that no cases of OHSS were reported.

2.2.4 The American Approach

The “American approach” as it is now commonly referred to, focuses on more intensive progesterone and oestradiol support of the luteal phase to minimise OHSS in high responders. One of the most cited studies in this area is a randomised control trial of 59 patients who were deemed at high risk of OHSS (*Engmann et al., 2008)*. The patients were randomised to either a GnRH-antagonist protocol and trigger with GnRH-agonist, or dual pituitary suppression (oral contraceptive pill and GnRH-agonist) followed by controlled ovarian stimulation and trigger with hCG. Luteal phase supplementation was started at oocyte retrieval + 1 day and consisted of 50mg IMI progesterone and three oestradiol patches (0.1mg each) on alternate days. This was continued until 10 weeks of gestation. Serum progesterone and oestradiol levels were measured on the day of oocyte aspiration (OA), OA + 7 days, and weekly thereafter. If the oestrogen levels were below 200pg/ml (734pmol/L), the patches were increased to four or oral oestrogen was added. If the progesterone levels fell below 20ng/ml (64 nmol/L), the dose of progestogen was increased to 75mg intramuscularly, or vaginal progesterone was added. The GnRH-agonist trigger group had a positive pregnancy rate of 63.3% (vs 62.1%), clinical pregnancy rate of 56.7% (vs 51.7%), and ongoing pregnancy rate of 53% (vs 48%). There were no cases of ovarian hyperstimulation in

the GnRH-agonist trigger group, versus 10 out of 29 patients (13.9%) in the hCG group. Critics of this study mention the higher level of LH in patients with polycystic ovarian syndrome (in the hCG trigger group), thus more studies in normo-gonadotrophic women are warranted.

The above study is often contrasted to the work done by *Babayof et al* in 2006 which showed an ongoing pregnancy rate of 6% and early pregnancy loss rate of 80% in the GnRH-agonists triggered group. It is important to note that the study by *Babayof* included 13 and 15 patients in the hCG and GnRH-agonist trigger group respectively. Intramuscular progesterone (Gestone 50 mg) was started 36 hours after the oocyte retrieval and increased to 100mg if the serum progesterone levels dropped below 40nmol/l. Estrogen was only added in the form of 4mg of Estrofem if the serum concentration dropped below 200pmol/L. Out of the four pregnancies in the hCG trigger group, two ended in early miscarriage. Five pregnancies were documented in the GnRH-agonist trigger group, of which two ended in early miscarriage, and two were biochemical pregnancies.

A clinic in Vietnam published their retrospective data on implantation, clinical pregnancy, ongoing pregnancy and live birth during a transition from hCG to GnRH-agonist triggering in high risk patients (*Iliodromiti et al., 2013*). In total, their study consisted of 620 cycles (363 GnRH-agonists triggers and 257 hCG triggers). The luteal phase was supported with daily oestrogen 6mg and 50mg of intramuscular progesterone (Gestone) PLUS 90mg vaginal micronised progesterone (8% Crinone) twice daily. The hCG group was supported with the same daily 8% vaginal Crinone gel regimen (until 7 weeks gestation), but 22 (8.6%) patients had fresh transfers cancelled due to OHSS symptoms. The patient characteristics between the two trigger groups were similar, including antral follicle count, AMH, age and BMI. The percentage of positive pregnancy tests was lower in the GnRH-agonist trigger group (36.9% vs 43.5%), but the clinical pregnancy rate and live-birth rates were similar in both groups, at 30% and 29% respectively. Interestingly, the miscarriage rate was lower in the GnRH-agonist trigger group (1.4% vs. 5.8%) – this may have been due to the longer progesterone support in this group. Unsurprisingly, 18 cases of OHSS were reported in the hCG group.

An alternative approach has been to freeze all embryos or oocytes in GnRH-agonist triggered cycle and transfer them in a frozen cycle to avoid the effect of the trigger on the luteal phase. A study published by *Imbar et al* in 2012 compared the outcomes of fresh and frozen-thawed cycles. This study reviewed 40 frozen-thawed transfers (after GnRH-agonist trigger) and 70 fresh transfers. The patients were all at high risk of OHSS having either low BMI, prior high response to ovarian stimulation or polycystic ovarian syndrome. The luteal phase was supported with 50 mg intramuscular progesterone (Gestone) and 6mg of oestrogen (Estrofem) daily, from the day of retrieval until 10 weeks of gestation. All transfers consisted of day 2 or 3 embryos. The frozen cycle endometrium was prepared with the same oestrogen regime, but additionally utilized 400mg of vaginal progesterone (Uterogestan) twice daily. The live birth rate was 27.1 % in the fresh cycle, compared to 20% in the frozen cycle (RR 1.36, 95% CI 0.65-2.81). The implantation rates and pregnancy rates were similar in both fresh and frozen embryo cycles. The frozen embryo transfer group had a higher miscarriage rate (15%) in comparison to a 10% miscarriage rate in the fresh embryo transfer group. The authors argued that considering some units may not have access to freezing or high survival rates when thawing embryos, this study proved the concept that the luteal phase could be sufficiently rescued in GnRH triggered cycles.

2.2.5 The Meta-Analyses

One of the first meta-analyses assessing outcomes in GnRH-agonist triggered cycles was published in 2006, consisting of three randomised controlled trials and 275 patients (*Griesinger et al., 2006*). Of importance is that standard luteal phase support was used in all three studies, and two were stopped before completion (*Humaidan et al., 2005; Kolibianakis et al., 2005 – discussed above).* The clinical pregnancy rate in the GnRH-agonist trigger group was substantially lower (7.9%) in comparison with the hCG group (30.14%, P = 0.02). No cases of OHSS were reported in two of the three included studies and the third did not record this variable.

A subsequent meta-analysis of randomised control trials was published after 2006 as a part of the Copenhagen GnRH-Agonist Triggering Workshop Group *(Humaidan et al., 2010*). These studies were grouped together as they all combined either intensive progesterone and oestradiol support, luteal LH or LH-like supplementation. This meta-analysis found a 7% OHSS rate in the hCG trigger group with no cases reported in the

GnRH-agonist group. Furthermore, there were significantly fewer deliveries (26.3%) in the GnRH-agonist group, versus 32.6% in the hCG group. The authors concluded that modified luteal phase support resulted in similar pregnancy rates with no OHSS. In this study, the optimal luteal phase support was not conclusively decided upon.

A Cochrane review examining GnRH-agonist triggers (*Youssef et al., 2014)* was updated in 2014, but has been heavily criticized for including small heterogeneous studies, with varying or no luteal phase support (*Kol et al., 2015*). Seventeen randomised control trials were included with a total of 1847 patients. Four of these RCT assessed donor-recipient cycles. When these four studies were excluded, the GnRH-agonist trigger group had a lower live birth rate in fresh autologous cycles than hCG (OR 0.47, 95% CI 0.31-0.70). A lower risk of OHSS was found with GnRH-agonists (OR 0.15, 95% CI 0.05 to 0.47) in comparison to hCG use. Furthermore, the GnRH-agonist group had a lower ongoing pregnancy rate (OR 0.70, 95% CI 0.54 to 0.91) and a higher early miscarriage rate (OR 1.74, CI 1.10 to 2.75). These findings were not replicated in the donor-recipient cycles, and this led the authors to conclude that GnRH-agonist triggers “could be useful” in donor cycles, fertility preservation cycles, and with a freeze-all strategy. The published criticism on this paper (*Kol et al., 2015*) states that previous work had shown similar pregnancy rates among GnRH-agonist triggered patients in frozen and donor-recipient cycles, and that an inadequate luteal phase, rather than the trigger itself, was to blame for lower clinical pregnancy rates.

Due to further emerging research, *Haarh* and colleagues decided to conduct a new meta-analysis of existing data of trials utilising a modified luteal phase support regimen (*Haahr et al., 2017*). Five studies were eligible for inclusion. The live-birth data were obtained from 857 IVF cycles and was found to be 26% for GnRH-agonist triggered cycles and 28% for those triggered with hCG. A subgroup analysis of most recent studies with individualized luteal phase support indicated an equivocal live birth rate with an OR of 1.08 (95% CI, 0.72 to 1.62). When considering an hCG bolus on the day of retrieval as the only modification to luteal phase support, the pooled OR was 0.78 (95% CI 0.52 to 1.18). The rate of OHSS was also assessed and graded according to criteria established by Humaidan in 2016 (*Humaidan et al., 2016*). All cases were described as moderate and late-onset. Although the rate of OHSS was lower in the

GnRH-agonist trigger group (0.9% vs 1.7%), this was not statistically significant. The clinical pregnancy rate was reported in all 859 cycles. This was similar in the GnRH-agonist trigger with luteal phase support (33%) and hCG (34%) trigger groups, with an OR of 0.99 (95% CI 0.74 to 1.32). Only two studies reported the ongoing pregnancy rate with an OR of 0.95 (95% CI 0.59 to 1.53) for GnRH-agonist triggered cycles. The miscarriage rate was calculated for 281 clinical pregnancies. The GnRH-agonist group had a higher miscarriage risk with an OR of 1.85 (95% CI 0.97 to 3.54). This is more tangibly described as 20% in the GnRH-agonist trigger group, versus 12.5% in the hCG group. The authors did not comment on the low level of evidence, or the fact that the study by *Andersen et al* (2015) significantly influenced the overall result.

The data outlined above illustrates the conflicting nature of the current body of evidence on the effect of GnRH-agonists for oocyte maturation on pregnancy and early pregnancy loss rates. It is clear that there is still no consensus on the ideal luteal phase support in these cycles, and that further well-designed, prospective studies, are needed to assist in answering these questions.

COMPARISON OF PREGNANCY RATES IN GnRH-AGONIST AND hCG TRIGGERED IVF CYCLES

3. AIMS AND OBJECTIVES

3.1 OBJECTIVES

a) To assess the difference in pregnancy rates between hCG and GnRH-agonist triggered patients in autologous IVF cycles.
b) To study the demographics of patients undergoing IVF treatment at Cape Fertility Clinic.

3.2 AIMS

To determine the difference in pregnancy rates between hCG-triggered and GnRH-agonist triggered IVF cycles, the latter with high dose progesterone luteal phase support.

4 METHODS

4.1 STUDY DESIGN

This was a retrospective clinical study.

4.2 STUDY POPULATION AND STUDY SETTING

Patients who presented to Cape Fertility, a private fertility treatment centre in Cape Town, South Africa from September 2016 were included for a period of one year from 01 September 2016 to 31 August 2017. The reason for this start date was due to a change in embryo culture medium (effective 01 September 2016), the concern being a change in culture media may have positively influenced outcomes between the groups. Only patients who underwent controlled ovarian stimulation and fresh transfer of their own oocytes were included. The patients were treated by three reproductive

medicine specialists and one reproductive medicine fellow (under supervision). The groups for analysis were selected based on the ovulation trigger used in the IVF cycle (hCG 250 ug subcutaneously, versus GnRH-agonist, 4mg subcutaneously in divided doses). The *progesterone supplementation* used in the GnRH-agonist trigger group was intramuscular progesterone (Gestone 100mg daily) while that utilised in the hCG group was 90mg vaginal progesterone daily (Crinone 8%).

4.3 STUDY DURATION

Patient data was collected between September 2016 and August 2017. The number of patients needed for analysis in the study was determined by a power calculation (see below).

4.4 SAMPLE SIZE

Null hypothesis: There would be no difference in the pregnancy rate per embryo transfer when either a GnRH-agonist or hCG was used for oocyte maturation.

Alternative hypothesis: The GnRH-agonist trigger group would have a 20% lower pregnancy rate even with improved luteal support.

Calculation of difference in pregnancy rate: Multiple studies, including a Cochrane review *(Humaidan et al., 2005; Kolibianakis et al., 2005; Griesinger et al., 2006; Youssef et al., 2014)* suggest a decrease in pregnancy rates of between 20% and 50% when a GnRH-agonist is used for ovulation maturation with standard IVF luteal support equivalent to 90mg of Crinone 8% vaginal progesterone gel. These studies suggest that the luteal phase cannot be rescued, and a “freeze all embryos” approach with a view to delayed embryo transfer is the best solution to improve the reduced pregnancy rate. It is not proven that using higher progesterone doses can rescue the luteal phase, but it may minimize the difference in pregnancy rates seen between the two ovulation induction regimens. It can be assumed that at least a 20% decrease in pregnancy rate per transfer will be observed when a GnRH-agonist trigger is used, even in the presence of higher progesterone administration in the luteal phase.

For the power calculation, the alpha value was set at 0.05. The beta value was set at 0.2. Power was set at 80%.

Anticipated pregnancy rate per embryo transfer in the hCG group: Previous years IVF success rate audits performed at the clinic indicated a 40% pregnancy success rate per cycle.

Analysis of ratios of patients receiving hCG or a GnRH-agonist for oocyte maturation: The patient folders were collected over an equal duration of time as fewer patients required GnRH-agonists in their cycles. This introduced a natural time-related skew in recruited numbers between the two trigger types. The number of patients in each group was therefore expected to be different, and to omit patients from one group would not have reflected accurate clinical practice, and may have biased results. Based on the clinic's practice data, it was anticipated that there would be three times as many patients in the hCG group as there were in the GnRH-agonist trigger group.

Based on the power calculation, 181 patients were needed in the hCG triggered group, and 54 patients in the GnRH-agonist triggered group.

4.5 STUDY INCLUSION CRITERIA

1. All women presenting to the clinic for controlled ovarian stimulation and in-vitro fertilization using their own oocytes with hCG or GnRH-agonist triggers between September 2016 and August 2017.
2. Patients with hCG or GnRH-agonist triggers and luteal support consisting of either 90mg vaginal progesterone (Crinone 8%) or 100mg intramuscular progesterone (Gestone) respectively.
3. Patients with fresh embryo transfer cycles.
4. Embryos from donor/ testicular biopsy sperm, as the source of the sperm is unlikely to affect outcome according to trigger type.
5. Transfers of day 3 and day 5 embryos.
6. Patients less than 40 years of age.

4.6 STUDY EXCLUSION CRITERIA

1. Patients requesting pre-genetic screening.
2. Patients in whom a pregnancy test outcome could not be established.
3. Patients who were older than 40-years of age.
4. Patients who required donor oocytes, as they would not receive an oocyte maturation trigger.
5. Repeat cycles in the same patient.
6. Patients who received an alternative form of progesterone for luteal phase support in the GnRH-agonist group.

4.7 CLINICAL PROTOCOL

All patients who chose to undergo treatment were screened via medical history, physical examination and pelvic ultrasound. Uterine cavity morphology, endometrial appearance, ovarian morphology and antral follicle count were noted on vaginal ultrasound. Routine infectious blood tests and endocrine markers were assessed. These included HIV screening, syphilis testing, Hepatitis B and C screening, Rubella immunity, thyroid function, prolactin levels, and an Anti-Mullerian Hormone level. Based on these findings, the doctors selected an individualised stimulation protocol for patients.

Patients who underwent IVF were stimulated with gonadotrophins. These may have been urinary-derived gonadotrophins (Menopur), or recombinant gonadotrophins (Pergoveris, Gonal-F). Ovulation was prevented with a GnRH-antagonist (Cetrotide). The patients were monitored via serial ultrasound scans until the follicles were of sufficient size (18-20mm) to induce oocyte maturation. The choice of the trigger type (hCG or GnRH-agonist Lucrin) was at the attending doctor's discretion. The total number of follicles in both ovaries were considered, as well as the patient's AMH and age. A GnRH-agonist was preferred when 12-15 follicles greater than 14mm were

present, the patient had an AMH suggesting excessive response to stimulation (> 2.56 ng/ mL), or the patient was under the age of 35-years. The GnRH-agonist trigger was administered as 40 (2mg) units subcutaneously 35 and a half hours, and 23 and a half hours before retrieval. hCG was administered 35.5 hours before planned retrieval in its recombinant form, Ovitrelle (250 ug subcutaneously).

Progesterone support was instituted on the day of oocyte retrieval. If a GnRH-agonist was used to trigger ovulation this was routinely supplemented with oestrogen (Estrapause 2mg tablets, two tablets twice daily). Progesterone support was provided in the form of daily intramuscular injection (Gestone 100mg). Luteal support was started on the day of the egg retrieval. Patients triggered with hCG were given vaginal gels (90mg 8% Crinone), once daily.

The embryo transfer was planned for either day 3 or day 5, depending on embryo growth, number, and superiority of development. If four or more embryos were at the 6-8 cell stage on day 3, the transfer was delayed until day 5. If there were less than 4 embryos at the 6-8 cell stage, this resulted in immediate transfer on day 3, unless the decision was made between the physician, embryologist and patient to delay transfer until day 5. Transfer was done under ultrasound guidance. In the case of positive pregnancy tests, luteal phase support was continued until 10 weeks gestation. This cut-off was chosen as many of the patients do not supply data beyond the completion of the luteal phase support at 10 weeks gestation, and first trimester screening results are not always performed in African countries where access to health care is poor. It is acknowledged that live-birth would have been the most ideal outcome. Excess blastocysts of AA, AB or BA quality were vitrified for future use.

4.8 DATA COLLECTION

Data were collected from existing patient folders by the primary investigator. The patient details were kept anonymous and only a 5-digit folder number was recorded. The patient's age, along with the type of ovulation trigger and progesterone support were recorded. The pregnancy test outcome per embryo transfer was recorded (a doubling in serum ßHCG levels 48 hours apart). Where no pregnancy test outcome was available, the patient was contacted to ask for the outcome.

4.9 DATA PROCESSING AND STATISTICAL ANALYSIS

Descriptive statistics were analysed using Prismm7 statistical software package. Numerical data were described using median and ranges, followed by testing of the data's distribution to inform the choice of statistics. Statistical analysis of the categorical data was performed using a chi-squared test. Analysis of continuous data was performed using an independent t-test or Mann-Whitney U test, depending on the population distribution for two comparison groups.

4.10 ETHICS

Ethics approval was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town under the number 189/2019.

Confidentiality was maintained by all involved in the study, as the stimulation protocol and pregnancy test outcomes were a part of the patient's confidential medical records. The research specific number, and not name, was recorded in the data set.

As the study was retrospective, no change in usual clinical practice occurred.

All of the data was collated and processed by the principal investigator, under the supervision of the study supervisors. All of the results were kept confidential.

4.11 EXPECTED OUTPUTS

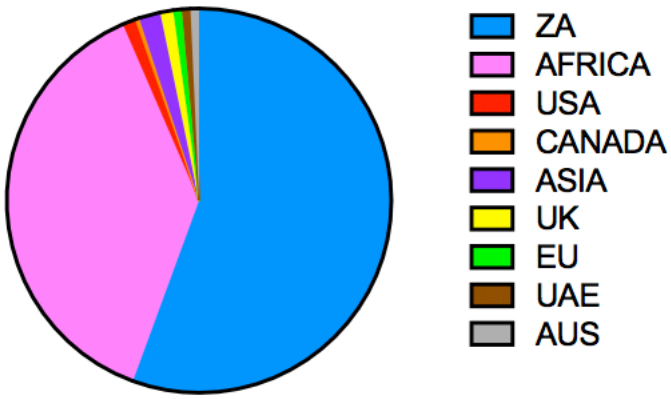
1. The study outcome may influence clinical practice through analysis of the data with respect to the best luteal support protocol in IVF cycles.
2. Outcomes may influence management of GnRH-agonist triggered patients.
3. A Masters of Philosophy (MPhil) for the principal investigator.
4. Publication of findings in a peer-reviewed journal.
5. Presentation of findings at a national or international conference.

5.0 RESULTS

A total of 284 autologous fresh transfer cycles were suitable for analysis for a period of a year between September 2016 and August 2017. Five patients (3 in the hCG group and 2 in the GnRH-agonist group) could not be contacted for follow up results, and were therefore excluded from the analysis. Thus, 196 patients in the hCG group, and 83 patients in the GnRH-agonist group were included for analysis.

Patient nationality for the study sample size is illustrated in Graph 1. The majority of patients were of South African origin (55.60%), with the second largest group comprising patients from the rest of the African continent (37.90% - predominantly from Angola). The remaining 6.5% were collectively from the United States of America, Canada, Asia, United Kingdom, Europe, United Arab Emirates, and Australia.

GRAPH 1: Patient nationality



Age and other ovarian reserve tests are shown in Table 1. The median age in the hCG trigger group was 34 years, and 33 years in the GnRH-agonist trigger group ($p < 0.0007$). The significant p-value with such a small age difference is a result of the use of the Mann-Whitney test. This test compares the means of the obtained values when ranked in order and not medians; i.e. this test does not compare the distribution of data in the two sets.

The antral follicle count (AFC) was available for 107 patients in the hCG trigger group and 55 patients in the GnRH-agonist trigger group. The median AFC was 10 follicles in the hCG trigger group, and much lower in comparison to 20 follicles in the GnRH-agonist trigger group ($p < 0.0001$).

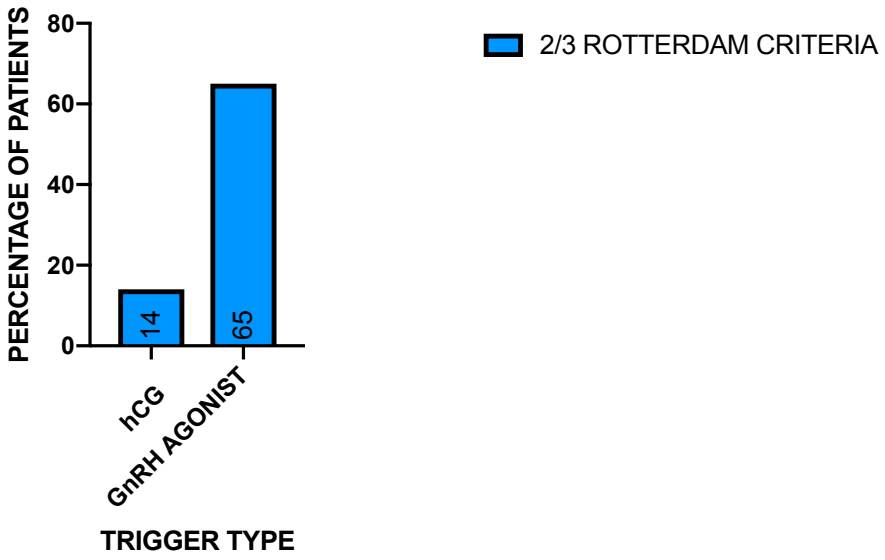
The AMH was available for 193 patients in the hCG group, and 80 in the GnRH-agonist group. The median AMH was lower in the hCG trigger group at 1.60 ng/mL, compared to 4.30 ng/mL in the GnRH-agonist trigger group ($p < 0.0001$).

TABLE 1: Markers of ovarian reserve

	hCG (n=196)	**GnRH (n=83)**	
	Range (Median)	**Range (Median)**	***p* - value**
Age (years)	24-40 *(34)*	24-40 *(33)*	0.0007
AFC	1-40 *(10)*	6-40 *(20)*	<0.0001
AMH (ng/mL)	0.09-20.91 *(1.60)*	0.97-25.03 *(4.30)*	<0.0001

Graph 2 shows the number of patients in each group diagnosed with polycystic ovarian syndrome as per the Rotterdam Criteria. Only 14% of the patients in the hCG trigger group met at least 2 of the 3 Rotterdam criteria, in comparison with 65% in the GnRH-agonist group.

GRAPH 2: Number of patients with 2/3 Rotterdam criteria present (by percentage).



The indication for IVF between the two trigger groups is tabulated in Table 2. Anovulation was the predominant IVF indication in the GnRH-agonist trigger group while decreased ovarian reserve (underlying reason not recorded in most folders) was the predominant IVF indication in the hCG trigger group. There was no significant difference in all other indications for IVF between the two trigger groups. Anovulation included all patients with irregular cycles regardless of PCOS diagnosis. Decreased ovarian reserve was classified as an AMH <1.14 ng/mL.

TABLE 2: Indication for IVF

	hCG	**GnRH-Agonist**	***p* - value**
Male Factor	46 (23.47%)	25 (30.12%)	0.2925
Tubal Factor	49 (25%)	18 (21.69%)	0.6462
Anovulation	*17 (8.96%)*	*28 (33.73%)*	*<0.0001*
Decreased Ovarian Reserve	*52 (26.53%)*	*2 (2.41%)*	*<0.0001*
Endometriosis	21 (10.71%)	3 (3.61%)	0.062
Unexplained infertility	6 (3.06%)	5 (6.02%)	0.3122
Converted cycle	2 (1.02%)	0 (0%)	>0.9999
Same-sex Relationship	2 (1.02%)	2 (2.41%)	0.5849
HIV Sero-discordance	1 (0.51%)	0 (0%)	>0.9999

TABLE 3: IVF ultrasound and laboratory parameters

TRIGGER	**hCG (n=196)**	**GnRH (n=83)**	
	Range (Median)	**Range (Median)**	***p* - value**
ET (mm)	5-21 *(10)*	6-15 *(10)*	0.4325
Follicles	1-17 *(8)*	9-25 *(17)*	<0.0001
Day Of OPU	10-24 *(14)*	11-17 *(15)*	0.1377
Oocytes	0-23 *(7)*	4-35 *(17)*	< 0.0001
Mature Oocytes	0-20 *(5)*	2-30 *(12)*	< 0.0001
Difference In Predicted Oocytes And Mature Oocytes	0-11 *(3)*	0-14 *(4)*	0.0274
Number Of Oocytes Fertilized	0-19 *(4)*	1-26 *(10)*	0.0001
Percentage Of Oocytes Fertilized (mean)	81.71%	80.94%	

The IVF sonographic and laboratory parameters between the two trigger groups are shown in Table 3. The median number of follicles, total oocytes, mature oocytes and fertilized oocytes were significantly higher in the GnRH-agonist trigger group. The median number of oocytes aspirated was 7 in the hCG group and 17 in the GnRH-agonist trigger group. There was significantly less mature oocyte yield per aspiration in the hCG trigger group (5) in comparison to the GnRH-agonist trigger group (12) $p < 0.0001$.

There was no difference in the minimum endometrial thickness (5mm and 6mm in the hCG and GnRH-agonist trigger groups respectively). The median endometrial thickness was 10mm in both groups ($p = 0.4325$).

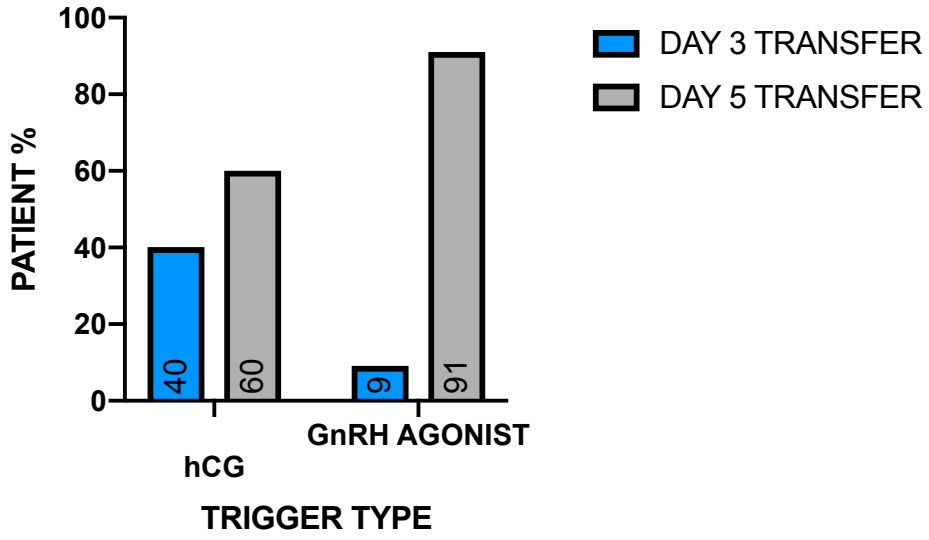
There was no difference in the timing of oocyte retrieval between the two groups. In the hCG trigger group the median day of retrieval was on day 14, in comparison to day 15 in the GnRH-agonist trigger group ($p = 0.137$).

The median reduction in the number of oocytes that were mature, in comparison to the number of follicles anticipated on ultrasound was calculated and found to be 3 fewer per aspiration in the hCG group, in comparison to 4 fewer in the GnRH-agonist groups.

The percentage of oocytes that fertilized was calculated for each group and was found to be no different in the hCG trigger group (81%), in comparison to the GnRH-agonist trigger group (80%).

Of the 183 patients who underwent an embryo transfer in the hCG trigger group, 74 (40.44%) had a transfer on day 3, versus 7 (8.64%) in the GnRH-agonist group. Conversely, more patients underwent a day-5 transfer in the GnRH-agonist trigger group (91.36%) when compared to the hCG trigger group (59.56%) - Graph 3 below. Thirteen patients in the hCG group did not have an embryo transfer in comparison to 2 patients in the GnRH-agonist group.

GRAPH 3: Day of embryo transfer by trigger group



A positive βhCG result was recorded in 41.84% of patients in the hCG trigger group and 49.40% of the GnRH-agonist group (Table 4).

A clinical pregnancy was demonstrated on ultrasound in 36.22% of patients in the hCG trigger group, and 43.37% of those in the GnRH-agonist trigger group (Table 4).

Pregnancy continued beyond ten weeks gestation in 33.16% of patients in the hCG group and 37.35% of the GnRH-agonist group. Ten weeks gestation was chosen as this data was consistently available for patients in the clinic.

In the hCG trigger group, 20.73% of patients suffered a miscarriage, in comparison to 24.39% in the GnRH-agonist trigger group(Table 4).

In summary, none of the observed differences in the serological pregnancy OR 1.36 (95% CI 0.81 – 2.29, p=0.2915), clinical pregnancy OR 1.35 (95% CI 0.812 – 2.29, p=0.2829), ongoing pregnancy OR 1.20 (95% CI 0.69 – 2.07, p=0.5819) and miscarriage rates OR 0.81 (95% CI 0.33 – 2.03, p=0.65) between GnRH-agonist and hCG triggered cycles reached statistical significance (Table 4).

TABLE 4: Pregnancy Outcomes

TRIGGER	**hCG** ***(%)***	**GnRH** ***(%)***	**Odds ratio GnRH** ***(95% CI)***	***p* - value**
BHCG +	82 *(41.84%)*	41 *(49.40%)*	1.36 *(0.81 – 2.29)*	0.2915
Clinical Pregnancy	71 *(36.22%)*	36 *(43.37%)*	1.35 *(0.812 – 2.29)*	0.2829
10 Weeks	65 *(33.16%)*	31 *(37.35%)*	1.20 *(0.69 – 2.07)*	0.5819
Miscarriage	17 *(20.73%)*	10 *(24.29%)*	1.233 *(0.49 – 3.02)*	0.6503

A sub-analysis of pregnancy outcomes comparing patients with more than 15 and less than 15 oocytes in the GnRH-agonist trigger group was performed (Table 5).

The groups were analysed according to positive pregnancy test, clinical pregnancy, and ongoing pregnancy at 10-weeks gestation.

There was no significant difference in serological, clinical and ongoing pregnancy rates between patients with more than 15 and less than 15 oocytes in the GnRH-agonist trigger group (Table 5).

TABLE 5: Subgroup analysis of oocyte number and pregnancy outcomes in GnRH-agonist triggered group

	GnRH-agonist <15 oocytes	**GnRH-agonist ≥15 oocytes**	**OR (95% CI) <15 oocytes**	***p* - value**
βhCG + (%)	57.14%	43.75%	1.71 *(0.703 – 3.92)*	0.2702
Positive pregnancy (%)	48.57%	39.58%	1.44 *(0.58 – 3.39)*	0.5027
10-weeks gestation (%)	42.86%	33.33%	1.50 *(0.63 – 3.51)*	0.4912

6.0 DISCUSSION

The introduction of the GnRH-antagonist short stimulation cycle has been one of the key developments in recent IVF history. This has allowed for the use of GnRH-agonists for ovulation induction with resultant reduced risk of OHSS in at-risk patients (*Haahr et al., 2017*). OHSS can be a life-threatening consequence of ovarian hyper-response to stimulation, with both serious health and financial implications. A reduction in the burden of OHSS with the use of GnRH-agonist trigger has not come without cost, with reports of reduced pregnancy frequency and higher miscarriage rates (*Humaidan et al., 2011*). Given this background, the aim of this study was to assess if the GnRH-agonist triggered luteal phase could be rescued with intensive luteal phase support. This study demonstrated that intensive steroidal luteal phase support consisting of combined estrogen and intramuscular progesterone likely contributed towards comparable serological, clinical and ongoing pregnancies between GnRH-agonist and hCG triggers. This challenges the fundamental consensus recommendation that a fresh embryo transfer should be discouraged in the same cycle, in these cycles. Yet still, fundamental questions remain: whether similar pregnancy rates can be achieved between GnRH-agonist and hCG triggered autologous IVF cycles, or whether a freeze-all embryo strategy should be advocated in cycles with GnRH-agonist trigger? Posed differently, it is whether the patient safety benefits in GnRH-agonist triggered cycles (in relation to OHSS reduction) can be maintained without compromising pregnancy outcomes, such as on-going pregnancy rates.

This study included 279 patients and to the author's knowledge is the largest study to assess use of this specific luteal phase support protocol i.e. 100mg of intramuscular progesterone and 8mg of oral estradiol. A similar study by *Engmann et al (2008)* included 59 patients randomised to either GnRH-agonist or hCG triggering. This study however used lower doses of intramuscular progesterone (50mg) and had higher clinical pregnancy and ongoing pregnancy rates with GnRH trigger than our study. This may have been due to the study population which consisted of patients specifically at risk of OHSS. Another study by *Babyoff et al* in 2006, which included a small sample size of 28 patients, also titrated intramuscular progesterone from 50mg to 100mg depending on serum progesterone levels. Similarly, oral estrogen was only

added if serum estradiol levels were decreased. In contrast to our findings, this study reported a low ongoing pregnancy rate of 6% and high early pregnancy loss rate of 80% in patients with GnRH-agonist trigger group. A retrospective Vietnamese study which included 620 cycles utilized the closest luteal support regimen to our study (*Iliodromiti et al., 2013).* In this study, the luteal phase of patients who received the GnRH-agonist trigger consisted of 50mg intramuscular progesterone plus 90mg of 8% vaginal progesterone twice daily, along with 6mg oral estrogen (lower than in our study). This study showed lower positive pregnancy test results in the GnRH-agonist triggered group (versus the hCG triggered group), but similar clinical pregnancy rates (30%) and live birth rates (29%) between the two groups.

Our study revealed that the majority of patients were South African (55.6%) or from the rest of the African continent (37.9%). To the author's knowledge, this represents the largest African study of luteal phase support in the context of GnRH-agonist triggered cycles.

While many meta-analyses have found worse pregnancy outcomes with GnRH-agonist triggers, our study found similar serological, clinical and ongoing pregnancy rates between GnRH and hCG triggered cycles. This may be partly explained by some good prognostic characteristics in this patient group, but it seems probable that the high dose luteal phase support may have been a contributor to better pregnancy outcomes, since similar studies used comparatively lower doses of luteal phase support.

Markers of ovarian reserve are used to assist identification of patients at risk of hyper-response to average doses of FSH, as well as identify those who may benefit from higher dosing schedules. It is well established that the age of a patient has an influence on ovarian reserve as well as oocyte quality, as these typically decrease with advancing age (*Humaidan et al., 2015b)*. The women who required a GnRH-agonist trigger for oocyte maturation were marginally younger. Although the age range was the same in both groups (24 – 40 years of age), the median age in the GnRH-agonist group was significantly one year younger. Furthermore, the interquartile ranges for age in the GnRH-agonist triggered group were much lower and this may explain the observed pregnancy rates in the GnRH-agonist group.

Both the antral follicle count and AMH values for patients triggered with a GnRH-agonist were higher. The median antral follicle count (20) in GnRH-agonist triggered cycles was double that of those with hCG triggers (10). The median AMH value (4.30 ng/mL) observed in GnRH-agonist triggered cycles, is within the predicted *excessive ovarian response* in laboratory reference ranges. In addition to differences in age between the two trigger groups, both AFC and AMH may explain the observed increased pregnancy rates in the GnRH-agonist trigger group, as this translates to higher follicle and oocyte yield in response to IVF stimulation. A retrospective study assessing the relationship between the number of oocytes retrieved, day 3 euploid embryos, and cumulative live-birth rates showed a positive correlation between all three factors (*Venetis et al., 2018)*.

Additionally, a larger proportion (35%) of patients in the GnRH-agonist trigger group satisfied the Rotterdam Criteria for the diagnosis of polycystic ovarian syndrome (PCOS). In contrast, only 14% of patients in the hCG trigger group satisfied 2 out 3 criteria. However, despite the diagnosis of PCOS, most of these patients in the hCG trigger group responded sub-optimally to stimulation and therefore were triggered with hCG.

The indication for IVF may also have been a factor contributing to the better prognosis (compared to previous findings) in the GnRH-agonist triggered group. The main indications for IVF in the hCG triggered group were decreased ovarian reserve (26.5%), tubal (25%), and male factor infertility (23.47%). Only 2% of patients in the GnRH-agonist triggered group had decreased ovarian reserve as an indication, in fact the leading indication for IVF in this group was PCOS-related anovulation. These results are hardly surprising as the hCG trigger group is likely to have a lower ovarian reserve (as indicated by ability to safely trigger with hCG), in comparison to the GnRH-agonist trigger group with a higher anovulatory PCOS population.

The nature of the stimulation protocol between the two trigger groups were assessed. As expected, only 18% of the GnRH-agonist group utilized additional LH in the form of HMG. Fifty-six percent of the hCG group utilized HMG. This may be explained by slightly (but significant) older age, lower markers of ovarian reserve and a poorer

prognosis in the hCG group. The HMG may be added to a stimulation protocol for additional LH, higher FSH dosing or in place of r-FSH to reduce costs, especially when a poorer prognosis is identified (*Humaidan et al., 2015b*). Of note, recombinant FSH and LH (*Pergoveris)* was not used in any cycles during the study period.

The endometrial thickness (ET) is one of the best ultrasound assessments of the endometrial response to the follicular hormonal milieu (*Gallos et al., 2018)*. The median endometrial thickness was the same in both groups (10mm). A retrospective analysis of 25 767 cycles reported that the ideal endometrial thickness to maximize live birth rates and minimize miscarriage rates was 10mm *(Gallos et al., 2018).* One patient in the hCG group had an outlying endometrial thickness of 21mm. When this value is excluded, the rest of the values were quite comparable, and differences in the median ET values between the two trigger groups remained non-significant. Of interest is that patients in the GnRH-agonist trigger group, despite a higher follicular response, did not demonstrate a significantly higher median endometrial thickness. This may imply that endometrial thickness is perhaps not the best surrogate of endometrial receptivity in IVF patients.

The number of follicles reported on ultrasound on the day of trigger varied greatly between the two trigger groups. On the day of trigger, the GnRH-agonist group had a significantly higher median of 17 follicles on ultrasound, in comparison to 8 in the hCG group. This result is again not unexpected due to the lower age, as well as increased antral follicle count and AMH in the GnRH-agonist group. Additionally, an increased follicle number is often the primary reason for choosing GnRH-agonists as an oocyte maturation trigger.

The median number of oocytes retrieved in the GnRH-agonist group was 17, higher than in the hCG group with a median of 10. Similarly, the number of retrieved mature oocytes was significantly higher in the GnRH-agonist triggered group (12 vs 5 with hCG). Calculated as a percentage of mature out of total oocytes retrieved, this translated to 78.29% and 75.55% respectively. These findings are similar to those of *Humaidan et al* (2005 & 2010) who reported a significantly increased number of mature oocytes in the GnRH-agonist trigger group (85.2% vs 81.5% in the hCG group). This finding can possibly be explained by the effect of the GnRH-agonist induced FSH

surge, which is similar to that of a natural cycle. Previous studies have shown that FSH induces LH receptors in the luteinizing granulosa cells which may enhance the functioning of the corpus luteum. FSH also appears to induce the resumption of meiosis (*Zelinski-Wooten et al., 1995; Yding Andersen et al., 1999; Yding Andersen et al., 2005)*, which may increase the number of mature oocytes.

Although the number of oocytes showing signs of fertilisation were different between the two groups, the percentages of fertilized oocytes were similar (81.7% in hCG and 80.9% in GnRH-agonist trigger group). This concurs with the initial study by *Humaidan et al (2005)* that showed that despite a higher number of mature oocytes in the GnRH-agonist group, fertilization and cleavage rates were the same. Likewise, a study by *Kolibianakis et al (2005)* showed similar fertilisation rates between GnRH and hCG triggered cycles. It is still important to note that in the GnRH-agonist trigger group there were still more fertilized oocytes which progressed to transferrable embryos, and therefore potentially more blastocysts.

As this study was retrospective, the clinic protocol on the day of embryo transfer had already been implemented. Usually, if more than 4 embryos showing adequate progression (6-8 cell stage) on day 3 of assessment, the embryo transfer would be delayed to day 5 in order to choose the best quality embryos for transfer (by morphological assessment). Forty percent of hCG trigger patients had an embryo transfer on day 3, in comparison with 8% in the GnRH-agonist group. There was no significant difference in the pregnancy rates between day 3 (39%) and day 5 transfers (48.6%) in the hCG trigger patients. The numbers of patients with day 3 transfers in the GnRH-agonist trigger group were too small to warrant comparison. Existing literature is contradictory on whether pregnancy outcomes are better with a blastocyst transfer over a cleavage stage embryo transfer (*Glujovsky et al., 2016; Levi-Setti et al., 2018*. The larger amount of day 5 transfers in the GnRH-agonist group may reflect a larger cohort of “transferrable” quality embryos, and this may explain the higher pregnancy rates in this group.

Although comparable pregnancy rates with GnRH-agonist trigger were not an anticipated finding, if viewed as a “non-inferiority” finding, these results are similar to those reported in some studies. On the other hand, these results are dramatically

different from some of the original GnRH-agonist work done in the early 2000's (*Itskovitz et al., 2000; Bracero et al., 2001; Fauser et al., 2002; Humaidan et al., 2005; Kolinianakis et al.,2005)* which demonstrated low pregnancy rates, likely due to sub-optimal luteal phase support. It is perhaps not surprising (considering the previous discussion around inadequate corpus luteum function and importance of sufficient luteal phase support) that clinical and ongoing pregnancy rates were so low, with higher miscarriage rates with the use of GnRH-agonist trigger in these studies.

Our study's findings are most comparable to those of other studies where intensive progesterone and oestrogen support of the luteal phase, otherwise known as the "American Approach", was utilised. A study by *Engmann et al.* in *2008* demonstrated the elimination of OHSS risk with the use of GnRH-agonist triggers, without compromising implantation rates. In this study, 59 patients at high risk of OHSS were enrolled, and the stimulation and luteal phase support involved 50mg IMI progesterone and three oestradiol patches (0.1mg each) on alternate days until 10 weeks of gestation. Serum progesterone and oestradiol levels were measured on the day of oocyte aspiration (OA), OA + 7 days, and weekly thereafter. If the oestrogen levels were below 200pg/ml (734pmol/L), the patches were increased to four or oral oestrogen was added. If the progesterone levels fell below 20ng/ml (64 nmol/L), the dose of progestogen was increased to 75mg intramuscularly, or vaginal progesterone was added. This study reported high serological, clinical and ongoing pregnancy rates in both the hCG and GnRH-agonist triggered groups with no significant differences between the two groups. The study is significantly smaller than our study, and was performed in high risk patients (for OHSS) with an average of 18 and 20 oocytes in each group (81-83% mature oocytes, 71-74% fertilisation rate). The patients were also younger than in our study, and this could explain the higher success rates. It is important to note that their GnRH-agonist trigger group did not have inferior pregnancy success rates than the hCG group in this study, in agreement with our study findings.

The second similar study by *Iliodromiti et al* (2013) involved luteal phase support with 6mg of oral oestrogen and 50mg of intramuscular progesterone (Gestone) PLUS 90mg vaginal micronised progesterone (8% Crinone) twice daily in the GnRH-agonist trigger group. The hCG group was supported with the same 8% Crinone gel regimen (until 7 weeks gestation). The pregnancy rates were comparable between the two

trigger types (36.9% vs 43.5%). The clinical pregnancy rates were the same at 30%, and the livebirth rate was 29% in each arm. The similarity in the livebirth rates in the GnRH-agonist trigger group may suggest a higher pregnancy loss rate in the hCG group (5.8%) – a finding that has not been considered in many studies. The authors have suggested that this may be due to discontinuation of luteal phase support in the hCG arm at the time of pregnancy testing, and at 7 weeks in the GnRH-agonist group. The *Iliodromiti* study allowed for the transfer of three embryos, and the multiple pregnancy rate was higher in the hCG group (38.7% vs 25.9%). This may also have contributed to the higher pregnancy loss rate. This study provides further evidence that GnRH-agonist triggers do not necessarily result in inferior pregnancy success rates than hCG triggers provided there is adequate luteal phase support.

The debate around the best approach to luteal phase rescue is still ongoing. Although the current study protocol differs from the "European Approach" of hCG bolus or LH rescue of the luteal phase in GnRH-agonist triggered cycles, it is worth comparing pregnancy outcomes between the two approaches. The ensuing discussion addresses differences between luteal phase support regimen utilised in our study, the "European Approach" and pregnancy outcomes.

The initial hCG bolus study by *Humaidan et al* (2010) makes for an interesting comparison. Although this was a prospective randomised study comparing hCG and GnRH-agonist trigger with additional hCG bolus at 35 hours, the findings were relatively similar. It comprised 305 treatment cycles in which the mean age was slightly younger (31.5 and 30.9 years in the hCG and GnRH-agonist groups respectively). This study by *Humaidan et* al showed no difference in the percentage of mature oocytes and fertilisation rates between the GnRH-agonist and hCG trigger groups. Furthermore, there were no differences in the serological, clinical and ongoing pregnancy rates between the two trigger groups. The same study by *Humaidan et al* (2010) also noted a 4% non-significant difference in early pregnancy loss between the two groups. This is similar to the pregnancy findings of our study, and it seems there is not much difference whether hCG or high dose progesterone is utilized for luteal phase rescue.

Similar pregnancy rates were also seen in a study which aimed to find the ideal dose of additional hCG to rescue the luteal phase (*Castillo et al., 2010*). The authors cited clinical pregnancy rates of 47.7%, 42.6% and 39.4% in the three hCG bolus groups (1000 IU, 500 IU, and 250 IU respectively). Our study showed a clinical pregnancy rate of 43.37% in the GnRH-agonist group, which is slightly higher than two of the three hCG bolus groups above. Furthermore, no patients in our study were diagnosed with OHSS. Fifteen patients in the *Castillo et al* study were diagnosed with OHSS, 6 of whom were in the 1000 IU hCG bolus group. One can hypothesize that the luteal phase of the cycles in our study may have been as well rescued as those with a hCG rescue bolus, but without the undesirable consequences of OHSS.

From the above discussion, it can be surmised that intensive steroidal support of the luteal phase may equally rescue pregnancy outcomes in GnRH-agonist triggered cycles when compared with additional hCG bolus regimens, but without the increased risk of OHSS. As our study was retrospective and not powered to assess risk of OHSS, this would need to be further assessed in future studies.

There is a marked difference in pregnancy outcomes when comparing our study to some of the previously published meta-analyses. *Griesinger et al* published the first meta-analysis in 2006 which showed very low pregnancy success rates in the GnRH-agonist trigger group. The three studies, comprising 275 patients, instituted standard luteal phase support (vaginal progesterone and oral estrogen) and therefore the results were poor likely as a result of insufficient luteal support. Interestingly, the initial serological pregnancy rates were similar between the study groups, but the GnRH-agonist group suffered a high early pregnancy loss, suggesting inadequate luteal phase support. The Cochrane review published in 2014 included studies in which the luteal phase was insufficiently supported. The review combined studies with no luteal phase support, standard IVF luteal phase support (*Humaidan et al., 2005; Kolibianakis et al., 2005*) and modified luteal phase support (*Engmann et al., 2008*). This review has been highly criticised for this, and is therefore does not yield valid conclusions for comparison.

A meta-analysis performed in 2010 (*Humaidan et al., 2011*) found no difference in delivery rates between the two trigger groups when assessing randomized controlled

trials published after 2006 (26.3% in the GnRH-agonist group, 32.6% in the hCG group). All of these included studies had some form of modified luteal phase support. Our study did not assess delivery rates, but ongoing pregnancy rates at 10 weeks, and due to this limitation, relevant comparisons cannot be made.

The last meta-analysis by *Haahr et al* (2017) included 857 IVF cycles and showed a similar livebirth rate between the GnRH-agonist trigger (26.1%) and the hCG trigger groups (28.8%). Furthermore, when only isolating the most recent studies with individualized luteal phase support, the difference between two groups was minimal, with an OR 1.08 (95% CI 0.72 - 1.62). There was no difference between the clinical pregnancy rate (33% GnRH-agonist group; 34% hCG group) and the ongoing pregnancy rates (27.9% GnRH-agonist group; 28.7% hCG group). The miscarriage rates between the two groups were also similar.

When the GnRH-agonist trigger group in our study was sub-divided into two groups to assess outcome in relation to follicle numbers, the group with fewer than 15 follicles had better pregnancy outcomes. This finding has not been shown in other published studies. The prematurely terminated study by *Humaidan et al* (2013) (due to the death of one of the authors) also divided patients into two groups of 14 or fewer follicles, or 14 plus follicles. They then randomised patients to trigger with 5000 IU hCG or GnRH-agonist plus 1500 IU hCG at retrieval, and again at day 5 post retrieval when <14 follicles were seen. This study showed a higher pregnancy rate in the higher follicle group (48.1% vs 42.7%). The difference dissipated when assessing clinical pregnancy (35% vs 34.4%) and ongoing pregnancy (28.3% vs 29.6%). The authors commented that the study was not powered to assess reproductive outcomes (the main objective was assessment of OHSS), and that the results may have been a chance finding.

This does raise the question of why a higher number of follicles may result in lower pregnancy outcome in our study. Higher oocyte numbers may exaggerate some of the speculated negative effects of controlled ovarian stimulation on implantation and ongoing pregnancy by negatively affecting the endometrium. Multiple oocytes may result in higher estrogen and progesterone concentrations, which may in turn negatively impact genes thought to be involved in endometrial receptivity, as well as endometrial angiogenesis (*Wang et al., 2017*). Endometrial advancement from

premature progesterone elevations may also occur, resulting in asynchrony between the embryo and endometrium (*Wang et al., 2017*) due to change in endometrial proteinomics. This remains a fascinating area for further research.

Regardless of the underlying cause, strategies to overcome the effect of controlled ovarian stimulation on endometrial receptivity might involve implementation of an elective freeze-all embryo approach with transfer in a subsequent cycle in patients with high follicle numbers, or performing detailed endocrine assessments on the day of trigger administration with a view to delayed transfer in patients with a raised serum progesterone. A Cochrane review and meta-analysis found no difference in livebirth rates between fresh and frozen embryo transfer (FET), but the review included studies comprising patients with both poor and high ovarian response (*Wang et al., 2017*). Several studies have shown a higher implantation, ongoing clinical pregnancy, and live birth rates when high responders were offered an elective freeze-all cycle. A large matched cohort study found higher pregnancy rates in FET cycles than fresh transfers in a group of patients with an average of 21 oocytes retrieved (*Wang et al., 2017*). The ongoing pregnancy rate was higher in the freeze-all group (52.0%; 95% CI 49.4%–54.6%) in comparison to the fresh embryo transfer group (45.3%; 95% CI 42.7%–47.9%). The study also noted the effect of a raised progesterone on outcomes. In cycles with a progesterone level of >1.0 ng/mL on day of trigger, the odds ratio of ongoing pregnancy was 1.38 (1.11 - 1.71) in the freeze-all group. Another meta-analysis of randomised controlled trials performed a subgroup-analysis of fresh and elective frozen transfers according to oocyte yield (*Dieamant et al., 2017*). Five randomised controlled trials were included comprising 2728 cycles, and the authors concluded that a freeze-all strategy was most beneficial when 15 or more oocytes were retrieved. These findings were also echoed in a large randomised control trial comparing fresh and elective frozen embryo transfer cycles (*Chen et al., 2016*). The patients in this study had an average of 14 oocytes at retrieval, and the authors noted an increased livebirth rate in the frozen transfer group.

The actual number of oocytes required as a guide for elective frozen transfer is still unclear and differs between studies, as a recent report assessing high responders has shown (*Xu et al., 2017*). This single-center study of 1423 high responders showed that when 20 or more oocytes are retrieved, there was an increased odds ratio of clinical

pregnancy (OR = 2.46, 95% CI: 1.74–3.46, P < 0.001) and livebirth (OR = 2.27, 95% CI: 1.60–3.22, P < .001) in freeze-all cycles. The group which had 15 – 18 oocytes retrieved, had a smaller, non-significant increase clinical pregnancy and livebirth rates.

Finally, a retrospective study involving 82 935 cycles recorded in the Society for Assisted Reproductive Technology database analyzed cycles according to oocyte numbers and fresh or elective frozen transfer (*Acharya et al., 2018*). High-responders were classified as 15 or more oocytes retrieved. This group had a significantly higher clinical pregnancy rate in frozen cycles (61.5%) when compared to fresh transfers (57.4%). As the above studies were not done with the assessment of intensive steroidal luteal phase rescue in mind, further research is needed to elucidate whether the elective freeze-all strategy should be implemented in all high responders with over 15 oocytes after GnRH-agonist trigger for oocyte maturation. It is important to weigh the financial cost and time delay associated with a delayed embryo transfer, as well as the potential loss of viable embryos during the freeze/ thaw procedure against the potential gains in clinical pregnancy and livebirths rates, when evaluating the benefit of elective freeze-all cycles with GnRH-agonist triggers.

7.0 CONCLUSION

In conclusion, our study demonstrated that intensive steroidal luteal phase support consisting of combined estrogen and intramuscular progesterone likely contributed towards comparable serological, clinical and ongoing pregnancies between GnRH-agonist and hCG triggers. The fact that an acceptable pregnancy rate in GnRH triggered cycles was observed with this approach, challenges the fundamental consensus recommendation that a fresh embryo transfer should be discouraged in the same cycle, in these cycles. Although this study is retrospective, with the tandem limitations of quality and level of evidence, it remains one of the largest of studies including cycles with high dose luteal phase support, particularly on the African continent. Furthermore, miscarriage rates were only moderately increased in the GnRH-agonist group, suggesting that the duration and endometrial support provided by intensive steroid administration is likely adequate in comparison to patients triggered with hCG. Further robust multi-centre trials are needed to compare the high dose luteal support approach with immediate transfer, versus elective freeze-all approach in prospective randomised trials. In addition, a specific focus on the required number of follicles present at the time of GnRH-agonist trigger along with reliable markers of endometrial receptivity is needed to confirm whether these factors are relevant in determining the best time to transfer embryos.

8.0 STUDY LIMITATIONS

This study was retrospective.

The decision to use either ovulation trigger was at the discretion of the treating physician.

The underlying cause of infertility was not always factored into the analysis.

Patients with an hCG trigger were more likely to have a lower ovarian reserve on testing, a lesser response to stimulation, and more day three than day five embryo transfers due to clinic transfer policy. This would result in increased chances of more top-quality blastocysts for transfer in the GnRH-agonist group, and therefore an increased success rate.

Data was only available until 10 weeks of gestation in most patients. Live-birth rate is the preferred outcome to assess.

- Last name, First initial. (Year published). Article title. *Journal*, Volume (Issue), Page(s).

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