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2021
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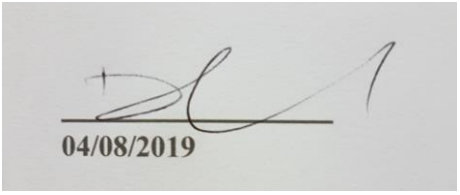
GIFT AND ZIFT: IS THERE A PLACE FOR THESE PROCEDURES IN A NEW ERA OF ASSISTED REPRODUCTION?

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This research reported is submitted to the University of the Witwatersrand Health Sciences Faculty in the submissible format in partial fulfilment of the degree of Master of Medicine in Obstetrics and Gynaecology in July 2019.

Declaration

I, Skye de Jongh declare that this work is original and contains no section copied in whole or in part from any other source, unless explicitly identified in quotation marks and with detailed, accurate referencing. I declare that this work has not been submitted to any other University for the purposes of a post graduate degree.



Dedication

I dedicate this Masters to my ever patient husband, Eben de Jongh.

Presentations

10/11/2017: Poster presentation at the Maternal Health Summit in Johannesburg

(Won second runner up)

14/06/2018: Oral presentation at Rahima Moosa Mother and Child Hospital

21/06/2018: Oral presentation at Vitalab Center for Assisted Conception

27/06/2018: Oral presentation at World Congress on Recurrent Pregnancy Loss in Madrid, Spain

06/09/2018: Poster presentation at The University of the Witwatersrand Research Day

List of Abbreviations

AMH: Anti-Mùllerian hormone

ART: Assisted reproductive technology

GIFT: Gamete intra-fallopian tube transfer

HIV: Human immunodeficiency virus

HSG: Hysterosalpingogram

ICSI: Intra-cytoplasmic sperm injection

IUI: Intra-uterine insertion

IVF-ET: In-vitro fertilization embryo transfer NILM:

Negative for intraepithelial lesion or malignancy

LSIL: Low grade squamous intraepithelial lesion.

PGS: Pre-implantation genetic screening

RIF: Repeated implantation failure

RPR: Rapid plasma reagin

TET: Thawed embryo transfer

ZIFT: Zygote intra-fallopian tube transfer

Abstract

The aim of this study is to document the incidence of successful pregnancy following gamete intra-fallopian tube transfer (GIFT) and zygote intra-fallopian transfer (ZIFT). It is a descriptive, retrospective study that took place at Vitalab Center for Assisted Conception in South Africa. Sixty three patients who underwent GIFT or ZIFT between January 2015 and March 2017 were included in the study. Each patient either underwent a GIFT or a ZIFT depending on their individual circumstances. The main outcome measures were; biochemical pregnancy, clinical pregnancy, miscarriage or ectopic pregnancy. The combined biochemical pregnancy success rate was 42.9% with a clinical pregnancy success rate of 29%. Interestingly, 50% of the subjects who previously had 100% aneuploidy, had biochemical pregnancies and 33% had successful clinical pregnancies. In conclusion, although GIFT and ZIFT exhibited a clinical pregnancy success rate of 33% versus the overall success rate of IVF of 58% at Vitalab in 2016, these techniques should be considered as options in those who have previously failed conventional IVF or ICSI more than twice. GIFT and ZIFT may also be considered in patients with recurrent 100% aneuploidy on PGS. Further research is needed to understand how one third of the patients who previously exhibited recurrent aneuploid embryos, achieved successful clinical pregnancies post GIFT and ZIFT

Acknowledgements

I would like to thank Vitalab Center for Assisted Conception for allowing me access to their patient data, as well as my supervisors for their on-going support and advice.

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Chapter 1: Literature review

1.1 INTRODUCTION

Infertility is defined as the inability to achieve a pregnancy after one year of regular, unprotected intercourse. It is a common problem affecting up to one in six couples. The causes of infertility can be divided into five groups; utero-tubal-peritoneal (30%), ovarian (20%), sperm migration (20%), male factor (30%), and unexplained (10%) (1).

The approach to management of infertility depends on the cause. In utero-tubal-peritoneal disease laparoscopic surgery is often attempted, followed by in-vitro fertilization (IVF). In those women affected by ovarian factor infertility, ovarian stimulation or egg donation is required prior to natural intercourse, intra-uterine insemination (IUI), or IVF in order to conceive. Sperm migration problems require assistance to enter the uterus therefore IUI or IVF can be used. Similarly, male factor infertility can be managed using IUI, IVF or donor sperm (2). The approach to unexplained infertility is to start with low-technology interventions and escalate therapy as indicated (3).

When faced with inadequate sperm count, morphology or motility, the treatment of choice is intra-cytoplasmic sperm injection (ICSI). This technique allows fertilization to take place by bypassing the initial steps of fertilization and injecting the sperm directly into the ooplasm (4). Once the oocytes are harvested they are placed in an incubator for 3-4 hours. They are then denuded, meaning that the cumulus cells surrounding the oocytes are removed using hyaluronidase and denuding pipettes. They are then assessed and grouped according to maturity and the presence of a polar body. If mature, the sperm is injected into the oocyte and fertilization occurs (5).

IVF is the current treatment of choice for tubal factor infertility, endometriosis and failed IUI. It involves the following: 1) ovarian stimulation, 2) ultrasound monitoring of the ovarian follicles and endometrial thickness, 3) oocyte harvesting, 4) fertilization, 5) in vitro culture of developing embryos and embryo transfer into the uterus trans-cervically (2). Embryos can also be frozen in liquid nitrogen and thawed at a later stage. Prior to embryo transfer, pre-implantation genetic screening (PGS) can be performed on the embryo on day five.

1.2 GIFT

Gamete Intra-Fallopian Transfer (GIFT) was first successfully performed by Ricardo Asch in Texas in 1984. The idea is that the GIFT procedure most accurately mimics the natural fertilization process and is therefore accepted by the Vatican. This allows the embryos not to be manipulated or discarded and fertilization takes place in-vivo (6).

GIFT always begins with a hysterosalpingogram (HSG) to analyze the patency and morphology of the fallopian tubes. Between 2001 and 2003 Kazan et al. showed that an HSG within six months of GIFT can benefit implantation rates by mechanical lavage, increasing cilliary activity and inhibiting macrophages (7).

Once patency is confirmed, ovarian stimulation is initiated in the female partner and sperm capacitation in the male partner, to optimize each parent's contribution. Once the eggs are fully matured they are harvested transvaginally. They are then placed, along with the capacitated sperm into a specific catheter and injected laparoscopically via the fimbrial end of the fallopian tube into the tube. Fertilization then takes place, leading to implantation into the uterus at the appropriate time and the development of a normal pregnancy (2).

GIFT is indicated in the treatment of infertility secondary to some ovarian and cervical diseases, as well as mild endometriosis, adhesions amenable to surgical endoscopic treatment and failed IUI. It has also been documented to be successful in some cases of Mùllerian dysgenesis (8). According to Rombauts et al. GIFT has a higher success rate in those with unexplained fertility. This is postulated that the cause is often an "egg" factor which then benefits from the tubal environment over the in-vitro environment (9). It is not useful in patients with tubal pathology, because at least one healthy fallopian tube is necessary to perform GIFT. It is also more invasive and more expensive than IVF. Other disadvantages include the increased risk for ectopic pregnancy, the risk of multiple gestations and the question of whether fertilization has actually occurred and whether the embryo is genetically normal or not (6). GIFT may be superior to IVF in cases of difficult transfers, poor laboratory conditions, shortage of media, religious objections or failed IVF (2).

Initial studies comparing pregnancy success rates between IVF and GIFT favored GIFT, for example according to the USA registry of 1992 a delivery rate of 16.8 percent per cycle was achieved using IVF compared to 26.3 percent in GIFT. However, most prospective studies have shown equivocal results when comparing the two. This has been postulated to be caused by better quality in-vitro culture conditions over the years, resulting in better pregnancy rates in IVF (10).

In terms of GIFT success rates there are a number of social factors that may have an impact on the outcome of the procedure; a study done by Klonoff-Cohen et al. in 2003 showed that alcohol consumed by female partners within a month of GIFT affected oocyte retrieval, pregnancy, and miscarriage rates. It also showed that alcohol consumed by male partners within a month of GIFT affected miscarriage and live birth rates (11). Similarly, consumption of more than 50mg of caffeine daily prior to the pregnancy negatively affected pregnancy rates and was associated with spontaneous abortion following GIFT (12). Karkoff-Cohen et al. also found a 40% reduction in oocyte retrieval and 46% reduction in live birth rates in those couples who smoked in the week of the GIFT with a 2.71 adjusted relative risk in those female partners who have smoked in their lifetime and 4.67 if they smoked for more than five years (13).

1.3 ZIFT

Later, in 1986 a patient with anti-sperm antibodies was successfully treated using Zygote Intra-Fallopian Transfer (ZIFT) otherwise known as Tubal Embryo Transfer (TET) by Devroey et al. The indications for ZIFT are severe male factor infertility, anti-sperm antibodies or repeated implantation failure (RIF) (14). The procedure is similar to GIFT, but instead of inserting gametes, fertilization takes place in the lab and a zygote is transferred after one day.

In order for a zygote to form, the sperm penetrates the oocyte leading to sperm-oocyte fusion. The oocyte is then activated and the male and female pro-nuclei begin to form. The pro-nuclei then gradually migrate to a central position within the oocyte, thus forming a spherical zygote containing two polar bodies and two pro-nuclei (15).

Levran et al. compared ZIFT with trans-cervical cleavage stage embryo transfer in 2002 in the treatment of RIF. ZIFT was found to have superior implantation and pregnancy

rates (1.4% vs. 3.7%). This is thought to be due to junctional zone contractions, expulsion of embryos and contamination of the catheter via the endo-cervical canal (16).

The American Society for Reproductive Medicine/ Society for Assisted Reproductive Technology registry compared the outcomes of IVF, GIFT and ZIFT in North America between 1991 and 1997; the conception rates were 30%, 35% and 35% respectively with delivery rates of 25%, 28% and 29% respectively. This data would suggest that GIFT and ZIFT were more successful than IVF; however, the number of procedures namely, 194554, 24166 and 7800 respectively negates that conclusion and suggests that the success rates were due to specialized population groups receiving each treatment. (The reason for the unpopularity of GIFT and ZIFT has been discussed above) (2).

Other possible benefits of ZIFT may be in the re-insertion of cryopreserved and then thawed zygotes. In a prospective study done by Frederick et al. in 1994 a pregnancy rate of 41% was achieved after intra-tubal transfer of thawed embryos and in 1995 Van Voorhis et al. found an implantation rate of 19% compared to 10% with intra-uterine transfer.

1.4 IVF FAILURE

Aneuploidy is common in IVF. A systematic review and meta-analysis of human embryos resulting from IVF in 2011 found that 73% of them contained some aneuploid cells. 25% of aneuploidy is due to errors occurring during meiosis and the remainder during mitosis. Some studies have suggested that ovulation induction increases the frequency of aneuploidy caused by meiotic errors. These errors can result in monosomy, which is usually incompatible with life unless the X chromosome is affected, causing Turner syndrome. Trisomy however, does support fetal growth but only trisomy 21 allows the fetus to reach adulthood. In a study done by Sagi et al. it was reported that haploid human parthenogenetic stem cells converted to diploid stem cells, suggesting the presence of chromosome correcting mechanisms (17).

In 1993 Cohen et al. suggested that women over the age of 40 were more susceptible to zona pellucida hardening due to culture media exposure. This was based on a study by Balmaceda et al. in 1992 where a pregnancy rate of 40.6% was achieved by GIFT

compared to 6% via ZIFT, suggesting that culture media exposed embryos had negative effects (10).

This is referred to as epigenetics. Epigenetics is a gene regulating activity that is primarily based on methylation of the DNA sequence. Alterations in the normal methylation status may affect normal development of the embryo and therefore gene expression leading to a phenotypically abnormal fetus. Epigenetic modifications have been found in embryos cultured in deficient media for extended periods of time, suggesting that poor media quality and over exposure to culture media may alter the genetic expression of an embryo (18).

In a study done by Yoshizawa et al in 2012 DNA methylation in the paternal pronucleus of rat zygotes was found to be reduced in IVF and ICSI conceived embryos versus in-vivo embryos. This was postulated to be due to the effects of culture media and superovulation. Human studies have been less successful due to ethical boundaries and have shown similar methylation levels and chromatin organization in IVF and ICSA with negligible differences between in-vivo and in-vitro twin outcomes (19).

There are many reasons IVF can fail and the cause could be maternal or embryological. Maternal causes include uterine anatomical abnormalities, sub-optimal endometrial conditions, thrombophilia and immunological conditions. Embryological causes include genetic abnormalities, laboratory stressors and technique-related errors (20).

Laboratory stressors can come in many forms; air pollutants, changes in pH and temperature, as well as light. Volatile organic compounds, microbes and perfumes in the air can harm the embryo and therefore measures, such as the use of activated carbon filters and positive pressure high efficacy particulate air need to be taken (21). Changes in pH can disrupt localization of mitochondria, increase glycolysis and alter embryo metabolism. According to a study done by Zader-Fox et al in 2008, lowering the pH in the culture media of an embryo resulted in fewer blastocysts, increased apoptosis and reduced fetal size (22). In a similar study in 2008 Hong et al. showed, that fewer blastocysts were found when the temperature of the incubator was decreased to thirty-six degrees Celsius (23).

Light has been found to affect cells directly by ionizing DNA and indirectly by oxidizing the media and changing it into a toxin. This indirect effect is caused by photo-oxidization of the oil in the media or in the sperm or oocyte membrane (24).

Epigenetics can also be in favour of the embryo. For example, in a study performed by Kervancioglu et al. in 1997, fertilization rates were found to be significantly increased in those wells containing human fallopian tube epithelial cell co-culture, specifically in infertility caused by a male factor, compared to those without co-culture (25).

Numerous studies have shown that embryo development in-vivo is supported by growth factors, cytokines and other embryotrophic factors produced by the oviductal epithelium. The epithelium also plays a role in removing deleterious substances from the tubal environment (26,27). In in-vitro studies using oviductal epithelial co-culture, the presence of the oviduct epithelium caused altered gene expression in the embryo and in turn, altered gene expression in the epithelium. This was confirmed when studying oocyte exposed oviductal epithelium to embryo exposed oviductal epithelium. A specific enzyme called Epoxide Hydrolase 1 present in the fallopian tube, has also been found to decrease reactive oxygen species levels and in so doing, enhance embryo development (28). The presence of these growth factors, cytokines and enzymes makes the fallopian tube an optimal microenvironment for embryo development and perhaps this is the reason that GIFT and ZIFT succeeded where IVF failed.

Studies using oviductal epithelial co-culture show improved quality of inner cell mass as well as improved fertilization and implantation rates. A study done by Hess et al. in 2011 showed that a group of chemokines called CXCL1,-2 and -3 are down-regulated in the presence of an embryo in the oviduct, thereby playing a role in accepting the embryo as a semi-allograft and the induction of angiogenesis. Other genes were up-regulated, resulting in protection from oxidative stress and maintenance of the embryo's structural and functional integrity (29). This study further demonstrated the protective function of the fallopian tube over that of the in-vitro environment, explaining the success of GIFT or ZIFT over IVF.

Chapter 2: Journal Article

GIFT and ZIFT Outcomes

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Conflict of interest:
SF de Jongh and AJ Wise- Nil
Y Unterslak- Employee at Vitalab Center for Assisted Conception Capsule:
In a retrospective, descriptive study GIFT and ZIFT had a biochemical pregnancy success rate of 42.9%. A third of the women with previous 100% aneuploidy had successful clinical pregnancies.

Gamete and zygote intra-fallopian tube transfer: Is there a place for these procedures in a new era of assisted reproduction?

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Abstract

Objective: This study aims to document the incidence of successful pregnancy following gamete intra-fallopian tube transfer (GIFT) and zygote intra-fallopian transfer (ZIFT).

Design: Descriptive, retrospective study.

Setting: Vitalab Center for Assisted Conception in South Africa.

Patients: Sixty three patients undergoing GIFT or ZIFT between January 2015 and March 2017.

Intervention: GIFT and ZIFT

Main Outcome Measures: Biochemical pregnancy, clinical pregnancy, miscarriage or ectopic.

Results: The combined biochemical pregnancy success rate was 42.9% with a clinical pregnancy success rate of 29%. Interestingly, 50% of the subjects who previously had 100% aneuploidy, had biochemical pregnancies and 33% had successful clinical pregnancies.

Conclusion: Although GIFT and ZIFT exhibited a clinical pregnancy success rate of 33% versus the overall success rate of IVF of 58% at Vitalab in 2016, these techniques should be considered as options in those who have previously failed conventional IVF or ICSI more than twice. GIFT and ZIFT may also be considered in patients with recurrent 100% aneuploidy on PGS. Further research is needed to understand how one third of the patients who previously exhibited recurrent aneuploid embryos, achieved successful clinical pregnancies post GIFT and ZIFT

Keywords: GIFT, ZIFT, Aneuploidy, Recurrent IVF failure, Pre-implantation genetic screening

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INTRODUCTION

Infertility is defined as the inability to achieve a pregnancy after one year of regular, unprotected intercourse. It is a common problem affecting up to one in six couples. The causes of infertility can be divided into five groups, namely: utero-tubal-peritoneal (30%), ovarian (20%), sperm migration (20%), male factor (30%), and unexplained (10%) (1). The treatment of choice will depend on the cause of infertility. IVF-ET is the current treatment of choice for tubal factor infertility, endometriosis and failed intra-uterine insemination. It involves the following: 1) ovarian stimulation, 2) ultrasound monitoring of the ovarian follicles and endometrial thickness, 3) oocyte harvesting, 4) fertilization, 5) in vitro culture of developing embryos and embryo transfer into the uterus trans-cervically (2). Many patients undergo multiple cycles of IVF that are unsuccessful; be it due to failed fertilization, aneuploidy, failed implantation, miscarriage or ectopic pregnancy. In this study gamete and zygote intra-fallopian tube transfer (GIFT and ZIFT) are investigated as possible alternatives.

Prior to undergoing GIFT or ZIFT a hysterosalpingogram (HSG) is performed to confirm tubal patency. Between 2001 and 2003 Kazan et al. showed that an HSG within six months of GIFT can benefit implantation rates by mechanical lavage, increasing ciliary activity and inhibiting macrophages (3).

Once patency is confirmed, ovarian stimulation is initiated in the female partner and sperm capacitation in the male partner, to optimize the contribution from each partner. Once the eggs are fully matured they are harvested transvaginally. They are then placed with the capacitated sperm into a specific catheter and injected laparoscopically via the fimbrial end into the tube. Fertilization then takes place, leading to implantation into the uterus at the appropriate time and the development of a normal pregnancy (2). Initial studies comparing pregnancy success rates between IVF and GIFT favored GIFT. In the USA registry of 1992, a delivery rate of 16.8 percent per cycle was achieved using IVF compared to 26.3 percent in GIFT. However, most prospective studies have shown equivocal results when comparing the two. This has been postulated to be caused by better quality in-vitro culture conditions over the years, resulting in better pregnancy rates in IVF(4).

The indications for ZIFT are severe male factor infertility, anti-sperm antibodies or repeated implantation failure (RIF) (5). The procedure is similar to GIFT, but instead of inserting

gametes, fertilization takes place in the laboratory with the use of intra-cytoplasmic sperm injection (ICSI) and a zygote is transferred after one day. The objectives of this study were primarily to document pregnancy outcomes following GIFT and ZIFT as explained above and secondarily to assess their success in those patients who previously had no embryos transferred due to 100% aneuploidy, as proven on pre-implantation genetic screening (PGS).

MATERIALS AND METHODS

Hypothesis

As a result of lack of funding in the state sector, most ART in South Africa is done in private facilities. Vitalab is one of those private facilities and over the years they have found a group of patients who have recurrent IVF failure. In response to this, the question was asked whether decreasing exposure to the laboratory would increase the pregnancy success in this group of patients.

The hypothesis was therefore that the incidence of aneuploidy was associated with laboratory exposure and that by doing GIFT and ZIFT, the laboratory could be avoided and pregnancy success rates would therefore increase.

Study design

A retrospective descriptive study was done at Vitalab Center for Assisted Conception in Sandton, Johannesburg. All of the patients who underwent GIFT or ZIFT between January 2015 and March 2017 were identified. All patients underwent an HSG followed by ovarian stimulation and oocyte harvesting (unless eggs were donated) and subsequent laparoscopic insertion of either gametes or zygotes into one of their fallopian tubes. Ethical approval was obtained from the Human Research Ethics Committee (medical). The clearance certificate number is M170611. Consent was obtained in the form of a pre-treatment consent form by Vitalab and anonymity was maintained by assigning file numbers to each patient.

Study population

All patients receiving GIFT or ZIFT between January 2015 and March 2017

Inclusion criteria:

- At least one patent fallopian tube
- GIFT or ZIFT between January 2015 and March 2017 Exclusion criteria
- Tubal pathology found at laparoscopy, not identified on HSG.

Sixty-five patients were recruited; one was excluded due to tubal pathology found at laparoscopy and one was excluded due to incomplete records.

Outcome measures

The primary end points were biochemical pregnancy (serum hCG > 5mIU/ml), clinical pregnancy (singleton, twin or triplet with a positive heart beat at seven weeks), serum hCG <5mIU/ml, miscarriage and ectopic pregnancy. Significance was defined as a p value of <0.05.

RESULTS

Table 1

Table 1 summarizes the demographics of the sample group of women. Of the 63 women, 75 % were over the age of 35. Although all races were recruited, the majority (82%) were white. Most women (71%) consumed some alcohol, but only 10% were smokers. The majority (73%) of women were nulliparous, however only 28.6% were nulligravid. In terms of ovarian reserve, 31.8% of patients were found to have low (< 1ng/ml) Anti-Mùllerian hormone levels (AMH). All patients with results had a negative RPR (n=42), were HIV negative (n=61) and were negative for Hepatitis B (n=62).

The causes of infertility were predominantly diminished ovarian reserve in 50.8%, followed by male factor infertility in 34.9%; the former, being the leading cause of infertility in women

over the age of 35 and the latter, the leader in women under the age of 35. Women under 35 years were significantly more likely to have male factor infertility as a cause of infertility versus over 35 years. Uterine abnormalities were found in 93.6% of women over the age of 35 and in 75% of women under 35 years. These abnormalities were often co-existent with hormonal abnormalities and not the primary cause of infertility.

Table 2

Table 2 summarizes the fertility history of the sample group. The mean number of previous forms of ART was three, with only two patients having had no previous ART. The most common form of ART previously attempted was IVF, followed by ICSI and thawed embryo transfer (TET). The majority of these attempts resulted in a serum hCG of less than 5mIU/ml units, although 9 successful pregnancies were noted. No euploid embryos were found in 12 patients. In terms of the PGS results, almost every type of monosomy and trisomy were identified once, with monosomy 16 and 22 occurring twice each in those under the age of 35 and Trisomy 13 and 21 occurring four and five times respectively in the over 35 years category. In the under 35 years age group only 10 aneuploid embryos were found, compared to 44 in the over 35 years age group.

Table 3

Table 3 summarizes the details of the GIFT and ZIFT process. GIFTs were done in 42.9% of cases, with the remaining 57.1% comprising of ZIFTs. Donor oocytes were used in 7.9% of cases and only 4.7% used donor sperm. The most common problems encountered with regards to sperm were hypo- and oligoteratozoospermia. Frozen gametes were used in 25% of cases. The mean number of gametes inserted was 5 and zygotes was 4.

Figure 1

The outcomes of this study show an overall biochemical pregnancy success rate of 42.9% with a clinical pregnancy success rate of 29%, with GIFT comprising 26% and ZIFT 33% of the total cases giving a p value of 0.5881 using a Fishers Exact test.
Of the successful clinical pregnancies; ten were singletons, six were twins and three were triplets. Approximately half of the interventions resulted in a serum hCG of less than five. The two “other” outcomes comprised of one instance where the ZIFT was cancelled as no oocytes were obtained post-stimulation and the other instance where only one oocyte was obtained

and the decision was taken to convert GIFT to IVF but there was no fertilization. The two patients with no history of ART both had a serum hCG of less than five post intervention. The 50% biochemical and 33% clinical success rate in the group with previous 100% aneuploidy was most interesting.

DISCUSSION

GIFT and ZIFT initially lost favor in the assisted reproductive community because it was more invasive and expensive and yielded similar results to IVF, without the benefit of confirmed fertilization and PGS. This study was undertaken mainly to review the outcomes of women in whom conventional IVF or ICSI had not proven successful at least twice in the past. GIFT and ZIFT were used as a second line in all but two cases and yielded a 29% clinical success rate. In view of this success, the invasiveness of a laparoscopic procedure and increased expense seemed justified. So why was GIFT or ZIFT successful where IVF was not?

In this study, 73% of women were nulliparous despite having had a mean of three previous ARTs and yet 29% had successful clinical pregnancies following GIFT or ZIFT. The majority of the women were white, RH positive and RPR negative, reflecting the demographic of Vitalab's clientele. Most had previously attempted IVF that had resulted in a negative serum hCG. Numerous studies have shown that embryo development in-vivo is supported by growth factors, cytokines and other embryotrophic factors produced by the oviductal epithelium. The epithelium also plays a role in removing deleterious substances from the tubal environment (6, 7).

In in-vitro studies using oviductal epithelial co-culture, the presence of the oviduct epithelium caused altered gene expression in the embryo and in turn, altered gene expression in the epithelium. This was confirmed when studying oocyte exposed oviductal epithelium to embryo exposed oviductal epithelium. A specific enzyme called Epoxide Hydrolase 1 (EPHX1) present in the fallopian tube, has also been found to decrease reactive oxygen species (ROS) levels and in so doing, enhance embryo development (8). The presence of these growth factors, cytokines and enzymes makes the fallopian tube an optimal

microenvironment for embryo development and perhaps this is the reason that GIFT and ZIFT succeeded where IVF failed.

Why did women who previously had no euploid embryos now have successful clinical pregnancies?

In this sample group, twelve women had previously had no euploid embryos to transfer during IVF, however six of those women had successful biochemical pregnancies and four had successful clinical pregnancies. All four of those babies had normal karyotypes at birth. It is hypothesized that by decreasing laboratory exposure, the rate of aneuploidy could be decreased. The reason for this hypothesis is that epigenetic modifications have been found in embryos cultured in deficient media for extended periods of time, suggesting that poor media quality and over exposure to culture media may alter the genetic expression of an embryo (9). In a study done by Yoshizawa et al in 2012 DNA methylation in the paternal pronucleus of rat zygotes was found to be reduced in IVF and ICSI conceived embryos versus in-vivo embryos. This was postulated to be due to the effects of culture media and superovulation. Human studies have been less successful due to ethical boundaries and have shown similar methylation levels and chromatin organization in IVF and ICSI with negligible differences between in-vivo and in-vitro twin outcomes (10). Data from Reprogenetics in the United Kingdom collected from trophoectoderm biopsies of 19719 embryos, suggested that the type of culture media (and brand) had an impact on the incidence of segmental mosaicism. It was found that continuous media was associated with a higher rate of mosaic embryos and portfertilization mitotic errors (11). This evidence supports the hypothesis.

The DNA of the embryos may also have been affected by other laboratory stressors. Aneuploidy can be caused by: 1) air pollutants, 2) changes in pH and temperature and 3) light. Volatile organic compounds, microbes and perfumes in the air can harm the embryo and therefore measures, such as the use of activated carbon filters and positive pressure high efficacy particulate air need to be used(12).

Changes in pH can disrupt localization of mitochondria, increase glycolysis and alter embryo metabolism. According to a study done by Zader-Fox et al in 2008, lowering the pH in the culture media of an embryo resulted in fewer blastocysts, increased apoptosis and reduced fetal size (13). In a similar study in 2008 Hong et al. showed, that fewer blastocysts were found when the temperature of the incubator was decreased to thirty-six degrees Celsius (14).

Light has been found to affect cells directly by ionizing DNA and indirectly by oxidizing the media and changing it into a toxin. This indirect effect is caused by photo-oxidization of the oil in the media or in the sperm or oocyte membrane (15). The oxygen concentration in the fallopian tube has been shown to be 5-6%, compared to the 21% oxygen concentration that human embryos are often cultured in. There are a number of transcription factors that are controlled by oxygen levels. These factors influence processes such as glycolytic metabolism, proliferation, migration, angiogenesis and other reproduction related processes. This vast change in oxygen exposure may alter the processes in in-vitro embryos, contributing to aneuploidy rates (16).

The decrease in laboratory stressors may not have been the only reason the aneuploidy rate decreased. The influence of the tubal environment may have had a corrective or preventative effect as well. Epigenetics can also be in favor of the embryo. For example; in a study performed by Kervancioglu et al. in 1997, fertilization rates were found to be significantly increased in those wells containing human fallopian tube epithelial cell co-culture, specifically in infertility caused by a male factor, compared to those without co-culture (17). Menezo et al showed in 2010 that 1.5-2 million repair processes are carried out in the early embryo in response to the effects of chemical insults like that of ROS. However, according to Menezo et al in their study in 2009, the oocyte's ability to repair these damages in the DNA does decrease with age and in the presence of smoking (18).

In contrast, our study showed 73.7% of the successful pregnancies were in women of advanced maternal age and two were smokers. In a study done by Sagi et al. it was also reported that haploid human parthenogenetic stem cells converted to diploid stem cells, again suggesting the presence of chromosome correcting mechanisms (9).

Limitations

The limitations of this study were the small sample size, the fact that it was retrospective and the fact that it was confined to one assisted conception centre. The external validity of the study would be limited due to the above-mentioned factors and therefore a larger study would be needed.

CONCLUSIONS

In conclusion, this study has found that GIFT and ZIFT are indeed viable alternatives to IVF in poor prognostic patients i.e. those with multiple previously failed ART, specifically due to 100% aneuploidy. Further randomized control trials are needed to investigate the relationship of IVF and ZIFT, following two failed ART. To truly compare the success rates, further research is also required on the micro-environment of the tube and how to emulate that environment in a laboratory setting.

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Table 1. Patient Demographics

Category	Classification	Under 35 years (n=16)	Over 35years (n=47)	p value
Gravidity	0	63(10)	38(18)	
	1	19(3)	45(21)	0.2862 (3.780)
	2	13(2)	13(6)	
	≥3	2(1)	4(2)	
Parity	0	94(15)	66(31)	0.0582
	1	0	28(13)	-5.689
	≥2	2(1)	6(3)	
Race	Asian	13(2)	9(4)	
	Black	2(1)	6(3)	
	Mixed race	2(1)	0	
	White	75(12)	85(40)	
AMH[a]	<0.5	13(2)	21(10)	
(n=46)	0.5-1.0	13(2)13(2)	13(6)13(6)	
	1.0-1.5	13(2)	9(4)	
	1.5-4.0	31(5)	26(12)	
	>4.0	2(1)	13(2)	
	Unknown	25(4)	28(13)	
Rh (n=60)	Positive	81(13)	83(39)	1.000*
	Negative	13(2)	13(6)	
Papsmear (n=59)	NILM[b]	88(14)	81(38)	0.1809 (-1.207)
	LSIL[c]	0	15(7)	0.1809 (-1.207)
Social Habits (n=51)	Smoking	2(1)	11(5)	1.0*
	Alcohol	69(11)	72(34)	1.0*

Note: [a]AMH measured in ng/ml, [b]NILM= Negative for intraepithelial lesion or malignancy, [c]LSIL= Low grade squamous intraepithelial lesion.

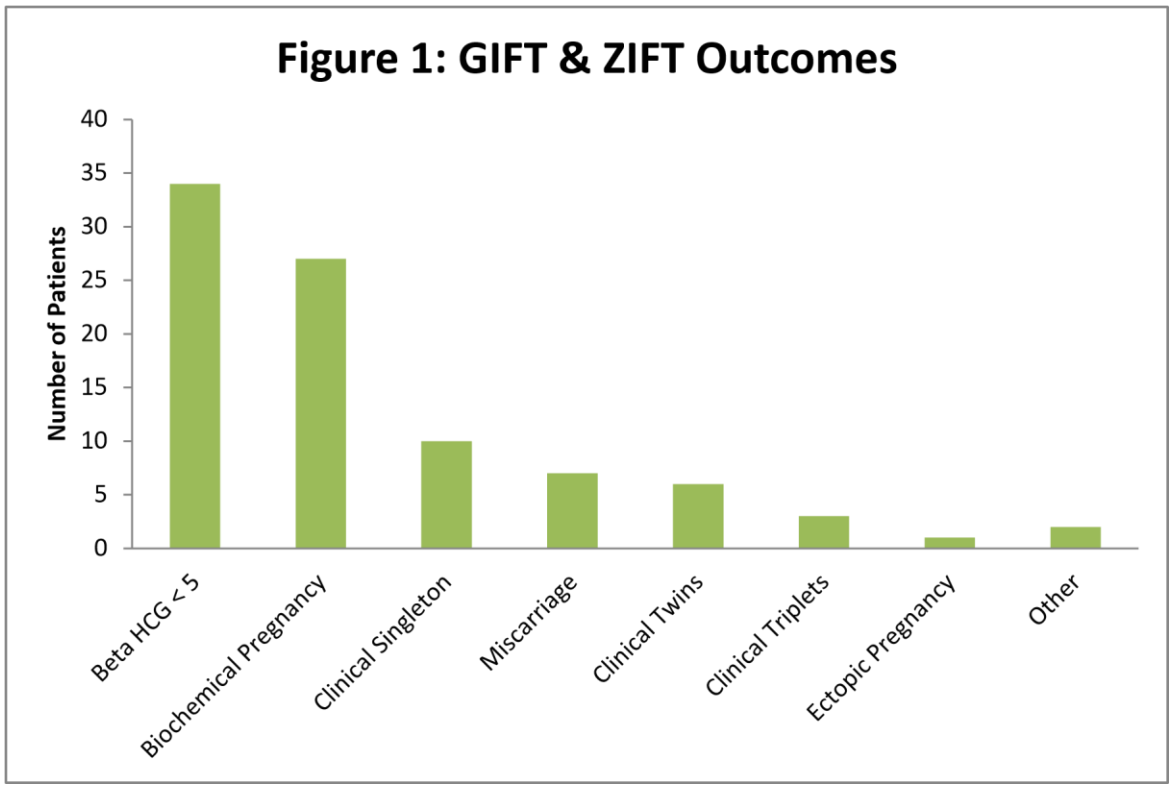
Table 2: Previous ART history

Category	Classification	Under 35 (n=16)	Over 35 (n=47)	Total (n=63)
Previous ART	IVF-ET	56(9)	55(26)	56(35)
	ICSI	63(10)	40(19)	46(29)
	TET	31(5)	40(19)	38(24)
	IUI	6(1)	13(6)	11(7)
	ZIFT	6(1)	13(6)	11(7)
	GIFT	13(2)	9(4)	10(6)
Previous outcome	Clinical pregnancy	6(1)	17(8)	14(9)
	Serum hCG <5	88(14)	77(36)	79(50)
	Miscarriage	25(4)	13(6)	16(10)
	Ectopic	0	0	0
	Frozen	19(3)	30(14)	27(17)
	No fertilization	13(2)	11(5)	11(7)
	No euploid embryo	6(1)	23(11)	19(12)
	Other	6(1)	0	2(1)
Previous PGS	Yes	31(5)	45(21)	41(26)

Note: values given as %(n)

Table 3: GIFT and ZIFT Details			
Category	Classification	Under 35 (n=16)	Over 35 (n=47)
GIFT OR ZIFT	GIFT	38(6)	45(21)
	ZIFT	63(10)	55(26)
Donor oocytes	No	100(16)	89(42)
	Yes	0	11(5)
Donor sperm	No	100(16)	94(44)
	Yes	0	6(3)
Semen analysis	Normal	31(5)	66(31)
	Asthenozoospermia	0	4(2)
	Azoospermia	6(1)	0
	Chemotherapy	6(1)	4(2)
	Hypospermia	25(4)	0
	Oligoteratozoospermia	19(3)	4(2)
	Previous Vasectomy	6(1)	13(6)
	Unknown	6(1)	9(4)
Fresh or frozen oocytes	Fresh	94(15)	89(42)
	Frozen	6(1)	11(5)
Fresh or frozen sperm	Fresh	28(13)	77(36)
	Frozen	19(3)	23(11)
Number of gametes inserted	1	6(1)	2(1)
	2	6(1)	0
	3	0	2(1)
	4	13(2)	4(2)
	5	0	11(5)
	6	6(1)	6(3)
	7	0	9(4)
	8	0	4(2)
	9	6(1)	2(1)
	13	0	2(1)
Number of zygotes inserted	1	0	2(1)
	2	6(1)	15(7)
	3	6(1)	6(3)
	4	19(3)	13(6)
	5	13(2)	13(6)
	6	19(3)	0
	7	0	6(3)

Note: values given as %(n)


Figure 1: GIFT & ZIFT Outcomes
Number of Patients
40
35
30
25
20
15
10
5
0
Beta HCG < 5
Biochemical Pregnancy
Clinical Singleton
Miscarriage
Clinical Twins
Clinical Triplets
Ectopic Pregnancy
Other

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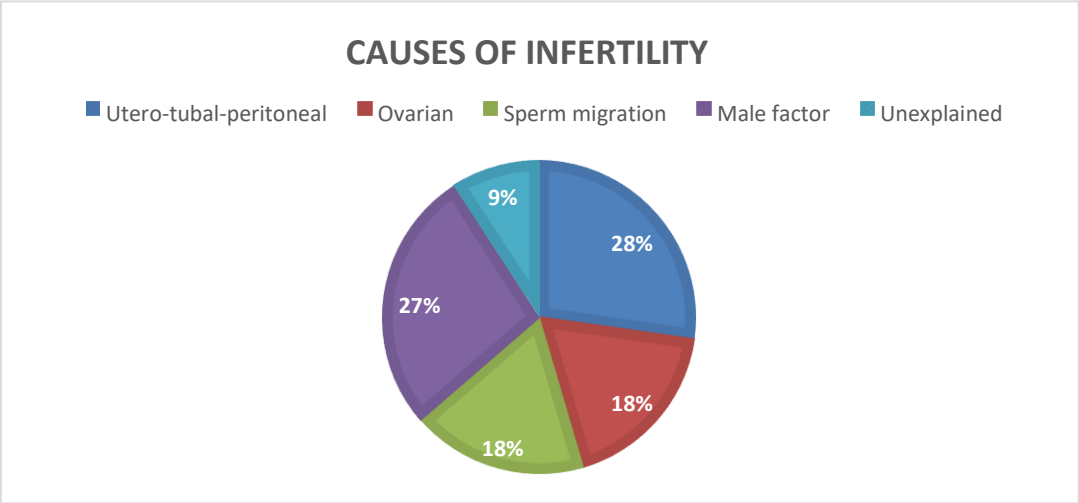
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Appendix A: Approved Research Protocol

1. Introduction

Infertility is defined as the inability to achieve a pregnancy after one year of regular, unprotected intercourse. It is a common problem affecting up to one in six couples. The causes of infertility can be divided into five groups; utero-tubal-peritoneal (30%), ovarian (20%), sperm migration (20%), male factor (30%), and unexplained (10%). [1]



The approach to management of infertility depends on the cause. In utero-tubal-peritoneal disease laparoscopic surgery is often attempted, followed by in-vitro fertilization (IVF). In those women affected by ovarian factor infertility, ovarian stimulation or egg donation is necessary, followed by natural intercourse, intra-uterine insemination (IUI), or IVF. Sperm migration problems require assistance to enter the uterus therefore IUI or IVF can be used. Similarly, male factor infertility can be managed using IUI, IVF or donor sperm.[2] The approach to unexplained infertility is to start with low-technology interventions and escalate therapy as indicated. [3]

When faced with inadequate sperm count, morphology or motility, the treatment of choice is intra-cytoplasmic sperm injection (ICSI). This technique allows fertilization to take place by bypassing the initial steps of fertilization and injecting the sperm directly into the ooplasm.[4] Once the oocytes are harvested they are placed in an incubator for 3-4 hours. They are then denuded, meaning that the cumulus cells surrounding the oocytes are removed using hyaluronidase and denuding pipettes. They are then assessed and grouped according to maturity and the presence of a polar body. If mature, the sperm is injected into the oocyte and fertilization occurs.[5]

IVF is the current treatment of choice for tubal factor infertility, endometriosis and failed IUI. It involves the following: ovarian stimulation, ultrasound monitoring of the ovarian follicles and endometrial thickness, oocyte harvesting, fertilization in the laboratory for five days and finally embryo transfer into the uterus trans-cervically, under sedation.[2] Embryos can also be frozen in liquid nitrogen and thawed at a later stage. Prior to embryo transfer, pre-implantation genetic screening (PGS) can be performed on the embryo on day 5.

GIFT

Gamete Intra-Fallopian Transfer (GIFT) was first successfully performed by Ricardo Asch in Texas in 1984. The idea is that the GIFT procedure most accurately mimics the natural fertilization process and is therefore accepted by the Vatican. This is due to the fact that the embryos are not manipulated or discarded and fertilization takes place invivo.[6]

GIFT always begins with an hysterosalpingogram (HSG) to analyze the patency and morphology of the fallopian tubes. Between 2001 and 2003 Kazan et al. showed that an HSG within six months of GIFT can benefit implantation rates by mechanical lavage, increasing cilliary activity and inhibiting macrophages.[7]

Once patency is confirmed, ovarian stimulation is initiated in the female partner and sperm capacitation in the male partner, to optimize each parent's contribution. Once the eggs are fully matured they are harvested transvaginally. They are then placed, along with the capacitated sperm into a specific catheter and injected laparoscopically via the fimbrial end of the fallopian tube into the tube. Fertilization then takes place, leading to implantation into the uterus at the appropriate time and the development of a normal pregnancy.[2]

GIFT is indicated in the treatment of infertility secondary to some ovarian and cervical diseases, as well as mild endometriosis, adhesions amenable to surgical endoscopic treatment and failed IUI. It has also been documented to be successful in some cases of Mùllerian dysgenesis.[8] According to Rombauts et al. GIFT has a higher success rate in those with unexplained fertility. This is postulated to be due to the fact that the cause is often an "egg" factor which then benefits from the tubal environment over the in-vitro environment.[9] It is not useful in patients with tubal pathology, because at least one healthy fallopian tube is necessary to perform GIFT. It is also more invasive and more expensive than IVF. Other disadvantages include the increased risk for ectopic pregnancy, the risk of multiple gestations and the question of whether fertilization has actually taken place and whether the embryo is genetically normal or not.[6] GIFT may be superior to IVF in cases of difficult transfers, poor laboratory conditions, shortage of media, religious objections or failed IVF.[2]

Initial studies comparing pregnancy success rates between IVF and GIFT favored GIFT, for example according to the USA registry of 1992 a delivery rate of 16.8 percent per cycle was achieved using IVF compared to 26.3 percent in GIFT. However, most prospective studies have shown equivocal results when comparing the two. This has been postulated to be caused by better quality in-vitro culture conditions over the years, resulting in better pregnancy rates in IVF.[10]

In terms of GIFT success rates there are a number of social factors that may have an impact on the outcome of the procedure; a study done by Klonoff-Cohen et al. in 2003 showed that alcohol consumed by women within a month of GIFT affected oocyte retrieval, pregnancy, and miscarriage rates. It also showed that alcohol consumed by men within a month of GIFT affected miscarriage and live birth rates.[12] Similarly, consumption of more than 50mg of caffeine daily prior to the pregnancy negatively affected pregnancy rates and was associated with spontaneous abortion following GIFT.[13] Karkoff-Cohen et al. also found a 40% reduction in oocyte retrieval and 46% reduction in live birth rates in those couples who smoked in the week of the GIFT with a 2.71 adjusted relative risk in those women who have smoked in their lifetime and 4.67 if they smoked for more than
five years.[14]

ZIFT

Later, in 1986 a patient with anti-sperm antibodies was successfully treated using Zygote Intra-Fallopian Transfer (ZIFT) otherwise known as Tubal Embryo Transfer (TET). The indications for ZIFT are severe male factor infertility, anti-sperm antibodies or repeated implantation failure (RIF).[14] The procedure is similar to GIFT, but instead of inserting gametes, fertilization takes place in the laboratory and a zygote is transferred after one day.

In order for a zygote to form, the sperm penetrates the oocyte leading to sperm-oocyte fusion. The oocyte is then activated and the male and female pro-nuclei begin to form. The pro-nuclei then gradually migrate to a central position within the oocyte, thus forming a spherical zygote containing two polar bodies and two pro-nuclei.[15]

Levran et al. compared ZIFT with trans-cervical cleavage stage embryo transfer in 2002 in the treatment of RIF: ZIFT was found to have superior implantation and pregnancy rates (1.4% vs. 3.7%). This is thought to be due to junctional zone contractions, expulsion of embryos and contamination of the catheter via the endo-cervical canal.[16]

The American Society for Reproductive Medicine/ Society for Assisted Reproductive Technology registry compared the outcomes of IVF, GIFT and ZIFT in North America between 1991 and 1997; the conception rates were 30%, 35% and 35% respectively with delivery rates of 25%, 28% and 29% respectively. This data would suggest that GIFT and ZIFT were more successful than IVF; however, the number of procedures 194554, 24166 and 7800 respectively negates that conclusion and suggests that the success rates were due to specialized population groups receiving each treatment. (The reason for the unpopularity of GIFT and ZIFT has been discussed above.)[2]

Other possible benefits of ZIFT may be in the re-insertion of cryopreserved and then thawed zygotes. In a prospective study done by Frederick et al. in 1994 a pregnancy rate of 41% was achieved after intra-tubal transfer of thawed embryos and in 1995 Van Voorhis et al. found an implantation rate of 19% compared to 10% with intra-uterine transfer.

IVF failure

In 1993 Cohen et al. suggested that women over the age of 40 were more susceptible to zona pellucida hardening due to culture media exposure. This was based on a study by Balmaceda et al. in 1992 where a pregnancy rate of 40.6% was achieved by GIFT compared to 6% via ZIFT, suggesting that culture media exposed embryos had negative effects.[10]

This is referred to as epigenetics. Epigenetics is a gene regulating activity that is primarily based on methylation of the DNA sequence. Alterations in the normal methylation status may affect normal development of the embryo and therefore gene expression leading to a phenotypically abnormal fetus. Epigenetic modifications have been found in embryos cultured in deficient media for extended periods of time, suggesting that poor quality and over exposure to culture media may alter the genetic expression of an embryo.[18]

In a study done by Yoshizawa et al in 2012 DNA methylation in the paternal pronucleus of rat zygotes was found to be reduced in IVF and ICSI conceived embryos versus in-vivo embryos. This was postulated to be due to the effects of culture media and superovulation. Human studies have been less successful due to ethical boundaries and have shown similar methylation levels and chromatin organization in IVF and ICSA with negligible differences between in-vivo and in-vitro twin outcomes.[19]

There are many reasons IVF can fail; the cause could be maternal or embryological. Maternal causes include uterine anatomical abnormalities, sub-optimal endometrial

conditions, thrombophilia and immunological conditions. Embryological causes include genetic abnormalities, laboratory stressors and technique related errors.[20]

Laboratory stressors can come in many forms; air pollutants, changes in pH and temperature, as well as light. Volatile organic compounds, microbes and perfumes in the air can harm the embryo and therefore measures, such as the use of activated carbon filters and positive pressure high efficacy particulate air need to be taken.[21] Changes in pH can disrupt localization of mitochondria, increase glycolysis and alter embryo metabolism. According to a study done by Zader-Fox et al in 2008, lowering the pH in the culture media of an embryo resulted in fewer blastocysts, increased apoptosis and reduced fetal size.[22] In a similar study in 2008 Hong et al. showed, that fewer blastocysts were found when the temperature of the incubator was decreased to thirty-six degrees Celsius.[23]

Light has been found to affect cells directly by ionizing DNA and indirectly by oxidizing the media and changing it into a toxin. This indirect effect is caused by photo-oxidization of the oil in the media or in the sperm or oocyte membrane.[24]

Epigenetics can also be in favour of the embryo. For example; in a study performed by Kervancioglu et al. in 1997, fertilization rates were found to be significantly increased in those wells containing human fallopian tube epithelial cell co-culture, specifically in infertility caused by a male factor, compared to those without co-culture.[25]

Aneuploidy is common in IVF. A systematic review and meta-analysis of human embryos resulting from IVF in 2011 found that 73% of them contained some aneuploid cells. 25% of aneuploidy is due to errors occurring during meiosis and the remainder during mitosis. Some studies have suggested that ovulation induction increases the frequency of aneuploidy

caused by meiotic errors. These errors can result in monosomy, which is usually incompatible with life unless the X chromosome is affected, causing Turner's syndrome. Trisomy however, does support fetal growth but only trisomy 21 allows the fetus to reach adulthood. In a study done by Sagi et al. it was reported that haploid human parthenogenetic stem cells converted to diploid stem cells, suggesting the presence of chromosome correcting mechanisms.[17]

2 Aim

The reproductive medicine specialists at Vitalab Center for Assisted Reproduction have found that, in women of advanced maternal age with decreased ovarian reserve, almost 100% of the embryos produced using IVF have some type of aneuploidy diagnosed by PGS. It was at this point that it was decided to attempt GIFT or ZIFT in this group of patients. The aim of this study is therefore, to assess the success of GIFT and ZIFT, following IVF and in the first instance and to hypothesize as to why the embryos have such high aneuploidy rates. We hypothesize that in cases of recurrent failed IVF associated with 100% aneuploidy, GIFT or ZIFT should be a viable option to achieve pregnancy.

3 Study Objectives

a. To evaluate the pregnancy rate in patients undergoing GIFT or ZIFT
    i. In those who have not undergone IVF previously ii.
    In those who have undergone IVF previously
b. To document the causes of infertility in these patients
c. To assess the frequency of multiple gestations as a result of GIFT and ZIFT
d. To compare pregnancy rates in GIFT and ZIFT

4 Methods

4.1 Setting

The Vitalab Center for Assisted Conception will be the setting for this study. Dr Jacobson, Dr Venter, Dr Gobetz and Dr Volschenk are all registered Reproductive Medicine Specialists with the Health Professions Council of South Africa and run the Centre along with Reproductive Medicine Assistant Dr Unterslak. The centre offers services to all nationalities of which 55% are South African, 37% African and 8% other foreign countries. They perform an average of 140 IVF procedures per month with one GIFT and ZIFT per month. In 2016 the biochemical pregnancy rates were as follows; IVF 58%, ICSI 65%, frozen embryo transfer 66% and PGS pregnancy rate 59%. The GIFT and ZIFT success rates are yet to be determined for 2016/7. In each case involved in the study, an HSG was done, followed by standard ovarian stimulation, egg retrieval, zygote formation in an incubator in ZIFT and laparoscopic insertion into the fallopian tube under general anaesthetic.

4.2 Study design

A descriptive study retrospectively analyzing the pregnancy outcomes of a defined group of patients undergoing GIFT or ZIFT

4.3 Study population

All patients receiving GIFT or ZIFT between January 2015 and March 2017

4.3.1 Inclusion criteria

- At least one patent fallopian tube
- GIFT or ZIFT between January 2015 and March 2017

4.3.2 Exclusion criteria

- Failed GIFT or ZIFT due to tubal pathology found at laparoscopy but undetected by HSG

4.3.3 Sample size

- All patients receiving GIFT or ZIFT between January 2015 and March 2017 will be included in the study. Approximately 30 GIFT and 37 ZIFT will be included.

4.4 Variables

- Reason for failed IVF
- Previous infertility interventions
- Cause of infertility
- Age
- Population
- Social: Alcohol, Smoking
- End points
    - Biochemical pregnancy (positive serum hCG at fourteen days)
    - Clinical pregnancy (positive fetal heart at seven weeks)
    - First trimester miscarriage
    - Ectopic pregnancy

4.5 Bias

- All cases were sourced and all procedures performed at Vitalab by different surgeons
- Indications for GIFT and ZIFT were not clearly defined before the study ➢ Retrospective analysis

4.6 Data collection

The data will be collected from Vitalab Center for Assisted Conception with assistance from the filing clerk. The eligible patient files will be selected using the inclusion and exclusion criteria and allocated a number so as to preserve anonymity. Thereafter the data will be transferred onto a data sheet (Appendix B) and analyzed in an excel spreadsheet.

5 Data analysis

Data will be analyzed with the help of a statistician from Wits using STATA software. Descriptive data in the form of continuous or categorical data will be analyzed to determine the prevalence of variables, means and medians. An association between these variables and the various outcomes will be analyzed using analytical statistics and logistic regression. These statistics will employ a p-value of 0.05, odds ratios and 95% confidence intervals. For normally distributed data, parametric tests such as Student's T test, ANOVA and Chi-square will be used. For data that is not normally distributed, non-parametric tests will be used.

6 Ethics

All patients will have signed a consent form prior to their procedure that allows their anonymous data to be used in future studies when they first consulted at Vitalab. (See Appendix D for Vitalab Consent form) Complete anonymity will be kept for all patients involved in the study by assigning each patient file a number. A non-disclosure agreement will be signed by the researcher with Vitalab Center for Assisted Conception to preserve that anonymity.

7 Timing

	March	April	May	June	July	Aug	Sept	Oct
Literature review	X							
Preparing protocol		X						
Protocol assessment			X					
Ethics application				X				
Collecting data						X		
Data analysis							X	
Writing up thesis								X
Writing up paper								X

8 Funding

All research costs will be funded by the researcher.

File Number:							
Age:							
Gravidity:	0	1	2	3	4	5	6
Parity:	0	1	2	3	4	5	6
Race:	Black	White	Asian	Coloured	Other	Unknown	
Social habits:	Smoking	Alcohol	None	Other	Unknown		
Cause of infertility:	Utero-Tubal-Peritoneal	Ovarian	Sperm Migration	Male Factor	Other	Unknown	
Donor or own gametes used:	Own Eggs	Own Sperm	Donor Eggs	Donor Sperm			
Number of failed IVF cycles received:	0	1	2	3	4	>4	
Cause of failed IVF:	**Maternal:**	Uterine Anatomical	Endometrial	Thrombophilia	Immunological	Other:	
	Embryological:	Genetic - Specify	Lab Stressors	Technique	Unkown	N/A	
Number of GIFT received:	0	1	2	3			
Number of ZIFT received:	0	1	2	3			
Number of gametes inserted:	0	1	2	3	4	5	6
Number of zygotes inserted:	0	1	2	3	4	5	6
Fresh or frozen gametes/ zygotes used:	Fresh Gametes	Fresh Zygotes	Frozen Gametes	Frozen Zygotes			
Outcome:	Biochemical Pregnancy	Clinical Pregnancy	Miscarriage	Ectopic			
	Specify Days post Procedure:						

Appendix B: Data collection sheet Appendix C: Patient History Form



"Setting the standards for Assisted Conception by combining the latest techniques with the highest levels of patient care"

www.vitalab.com
+27 (0) 11 911 4700

info@vitalab.com
ISO 15189 Accredited

PART 1: CONTACT INFORMATION

First name: _____ Middle Initial: _____ Last Name: _____

Age: _____ Date of Birth: _____ Occupation: _____

Home Address: _____

_____

City: _____ Province: _____ Postal Code: _____

Country: _____

Indicate which number to call or leave a message:

Home: _____ Work: _____ Cell: _____

Are you Married / Divorced / Other: _____

Spouse/Male First Name: _____Middle Initial: _____ Last Name: _____

Age: _____ Date of Birth: _____ Occupation: _____

Home Address: _____

_____

City: _____ Province: _____ Postal Code: _____

Country: _____

Indicate which number to call or leave a message:

Home: _____ Work: _____ Cell: _____

1

Contact Details:

Patient Email Address: _____

Patient Cell Phone Number: _____

Partners Cell Phone Number: _____

Partners Email Address: _____

GP Name _____

Contact details/Email Address: _____

Gynae Name _____

Contact details/Email Address: _____

PART 2: FEMALE MEDICAL HISTORY AND INFORMATION

Do you have any personal, ethical, or religious objections to any of our tests or treatments such as inseminations, in vitro fertilization, egg donation, sperm donation, masturbation to collect a semen sample, etc?

Yes / No: _____

How many months have you been having intercourse without using any form of birth control?

PREGNANCY SUMMARY

Total Number of **ALL** Pregnancies: _____

Number of Miscarriages (Less than 20 weeks): _____

Number of Ectopic/Tubal Pregnancies: _____

Number of Elective Terminations (Abortions): _____

Number of Full Term Deliveries: _____

Of these, how many were live births? _____

Any Pregnancies with Birth Defects? Yes / No. Explain: _____

_____

MENSTRUAL HISTORY

Menstrual cycle pattern (check all that apply): _____

Number of days between the start of one period to the start of the next period: _____

Dates of the 1st day of your last menstrual period: _____

If you do not have periods, at what age did you stop having them? _____

Do you have severe cramping or pelvic pain with your periods? _____

SEXUAL HISTORY

Do you have pain with intercourse? _____

Do you use lubricants (K-Y Jelly, etc) during intercourse? Yes what types? _____

Have you had any sexually transmitted diseases or pelvic infections? _____

PAP SMEAR HISTORY

When was your last pap smear? Date: - _____

Have you undergone any procedures as a result of an abnormal pap smear? _____

MEDICAL HISTORY

Are you allergic to any medications? _____

Please list: _____

Are you allergic to any foods (peanuts, seafood) _____

(Please list and describe reactions):

List current medication: _____

Do you have any medical problem(s) (Please list type and treatments below).

1. _____ Date: _____
2. _____ Date: _____
3. _____ Date: _____
4. _____ Date: _____

Have you had German Measles (Rubella) _____

Any other Childhood diseases: _____

SOCIAL HISTORY

Do you smoke cigarettes? - _____

Do you drink alcohol? - _____

Do you use marijuana, cocaine or any other similar drugs? Describe: _____

SURGICAL HISTORY

Have you had any surgeries? _____

(List all surgeries in chronological order)

Year Reason and Type of Surgery

1. _____

2. _____

3. _____

4. _____

5. _____

6. _____

Did you have any anaesthesia problems? – Describe: _____

FAMILY HISTORY

MEDICAL _____

GENETIC _____

PRIOR INFERTILITY TESTING AND TREATMENT

Have you had prior infertility testing or treatment elsewhere? - _____

Thyroid Test Date: _____ Results: _____

Hysterosalpingogram (HSG) Date: _____ Results: _____

Laparoscopy Surgery Date: _____ Results: _____

Hysteroscopy Surgery Date: _____ Results: _____

Abdominal Surgery Date: _____ Results: _____

Prolactin Blood test Date: _____ Results: _____

Previous IVF / ICSI / Insemination: - _____

PART 3: MALE MEDICAL HISTORY AND INFORMATION

Complete with your male partner if applicable.

Have you been evaluated by an urologist? _____

Do you have any children? _____

Have you had a semen analysis? _____

Do you have difficulty with erection? _____

Have you have any sexually transmitted diseases or pelvic infections? _____

Have you had a history of undescended testicles? _____

Did you have the mumps after puberty? _____

Have you had prior injury to your testicles requiring hospitalizations? _____

Have you had a vasectomy? Date: _____

Have you had surgery for varicocele repair? _____

Have you had hernia surgery? _____

Did you undergo any bladder or penis surgery as a child? _____

Have you had chemotherapy for cancer? _____

Are you allergic to any medication? _____

List and describe reactions: _____

List your current medications: _____

List any current medical problem (s) _____

Do you smoke? _____ How many _____

Do you drink alcohol? _____ How much _____

Do you use marijuana, cocaine, or any other similar drugs? _____

Describe _____

Appendix D: Patient Consent Form

FILE NUMBER: _____

COUPLE

CONSENT AND DECLARATION FORM FOR PARTICIPATION IN THE

ASSISTED REPRODUCTION PROGRAMME ("*THE PROGRAMME*") OF VITALAB INCLUDING:

A) IN-VITRO FERTILISATION AND EMBRYO TRANSFER - IVF

B) INTRACYTOPLASMIC SPERM INJECTION - ICSI

C) OTHER ASSISTED REPRODUCTIVE TECHNOLOGIES

1. Declaration:

1.1. We, declare that we are presently married to each other, which marriage still subsist.

1.2. We will sign a consent of confirmation that we indeed know each other in the capacity as indicated in this consent, *alternatively* our marital status is hereby confirmed and a copy of our marital certificate is attached to this consent marked "1".

1.3. We, further declare that we are of sound mind, both possessing full legal capacity to hereby authorise and direct the competent persons of the said institution, as envisaged in terms of Regulation 1 promulgated in terms of the National Health Act, Act 61 of 2003 (*"the Act"*) to administer and to treat us:

1.4. Full Name: _____

Date of Birth: _____

Identity Number: _____

Physical Address: _____

_____

("Recipient") AND

Initials: _____ _____

1.5. Full Name: _____

Date of Birth: _____

Identity Number: _____

Physical Address: _____
_____

Initials: _____ _____

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

3. We understand that the purpose of our participation in the programme is to attempt to become pregnant by In-Vitro Fertilisation and Embryo Transfer, Intracytoplasmic Sperm Injection, Gamete Intra-fallopian Transfer or other forms of Assisted Reproductive Technologies. The reason is that we have been unable to achieve a pregnancy because of conditions which have not been treatable by other currently available methods, or whose current success rates make these treatment modalities preferable.

4. We understand, from reading through the provided protocols and counselling, that the following is an outline of the process and procedures which will be followed during our participation in the programme, the relevant consents are attached hereto and form part of this consent : -

a) Administration of medication to assist ovulation.

b) Blood and ultrasound studies to monitor follicular development and quality.

c) Admission to the hospital for ultrasound directed ovum pick-up when the ovulatory process is at the appropriate stage as determined by the medical team, and in order to obtain as many eggs as possible from the ovaries.

d) The method for fertilisation of the eggs will be decided by the laboratory staff on the day of egg retrieval depending on the quality of the sperm specimen received.

e) If, for any reason, the eggs cannot be fertilised after collection, they will be frozen. The decision on how to fertilise them will then be made at a later stage.

f) Any changes that are made in the originally planned procedure will be discussed with the patients.

g) The chance of developing Ovarian Hyper Stimulation Syndrome is 0.3% for severe, 3-5% for moderate and 30% for mild. Appropriate action will be taken to minimize the risk, although it can never be eliminated.

h) <u>For In-Vitro Fertilisation (IVF) and Embryo Transfer (ET)</u>:

The eggs are mixed with the sperm to attempt to allow fertilisation to occur.

The fertilised egg(s) are transferred into a different medium for embryo development in the laboratory.

After several rounds of cell division, one or more embryos are transferred into the uterus by means of a small plastic tube.

i) For Intracytoplasmic Sperm Injection (ICSI):

Special preparation of the eggs to assess maturity and prepare them for insemination.

Specific preparation of the sperm to allow individual sperm selection.

Individual injection of each egg for attempted fertilisation.

The fertilised egg(s) are transferred into a different medium for embryo development in the laboratory.

After several rounds of cell division, one or more embryos are transferred into the uterus by means of a small plastic tube.

j) For Gamete Intrafallopian Transfer (GIFT):

Mixture of the eggs with sperm and placing the appropriate egg-sperm mixture into the Fallopian tube(s) by means of a plastic tube during a laparoscopy procedure performed at the same time as the egg collection.

5. We have been advised of all the reasonable known risks associated with this treatment, which have been fully explained to me (by the competent persons). (Laparoscopic mortality 0.05/1000 and major complications due to laparoscopy, requiring laparotomy, 3.8/1000).

6. We have been advised that there are no guarantees that a pregnancy will be achieved by our participation in this programme or that, if a pregnancy is achieved, a successful full-term pregnancy will ensue.

7. We understand the factors which might prevent a pregnancy or might not be conducive to carrying a pregnancy to full-term, include, but are not limited to, the following : -

a) The growth and maturation of eggs is not predictable, as such egg development might not occur in the monitored cycle, thereby precluding any reasonable attempt at obtaining suitable egg(s).

b) The attempt to obtain an egg(s) may be unsuccessful.

c) The egg(s) obtained might be unsuitable or too immature for fertilisation.

d) It might be difficult to produce a sperm sample.

e) Fertilisation of the egg(s) or their development into embryos outside the uterus could fail.

f) A laboratory accident might result in the loss of egg(s) or embryo(s).

g) Following the successful establishment of pregnancy, there is the possibility of miscarriage, ectopic (tubal) pregnancy or stillbirth.

h) Canalization of the Fallopian tube(s) might not be possible during a GIFT procedure.

8. We are aware of the reasonable risks and consequences of multiple pregnancies associated with these treatment modalities.

9. We understand that, should the pregnancy continue, there are no guarantees that birth defects will not occur.

10. We understand that there is no indication in scientific literature that the occurrence rate of any of the events stated in paragraph 7(g) or 8 is increased or decreased by these technologies.

11. We consent to the inherent risks associated with the programme, and all the possible outcomes associated with the programme, and/or pregnancy and/or birth and/or possible birth defects. We therefore waive any claim of any nature, whether jointly or severally liable against the authorised institution and/or the competent persons, and/or the employees, and/or agents and/or authorised representatives of the authorised institution in respect of any claim whatsoever including but not limited to a claim for damages and/or consequential damages, if any.

12. We understand that all embryos that do not grow appropriately to day 5 and all embryos that are not suitable for freezing will be discarded.

13. We give Vitalab permission to utilise these embryos that are not suitable for use or for freezing for training purposes in the laboratory before they are discarded.

Initials: _____ _____

14. We further consent to the freezing of any viable embryo(s) and their subsequent storage for possible future embryo transfer.

15. We understand that a separate consent form will be signed detailing the conditions of freezing. We further acknowledge that freezing is not a predictable technology.

16. We understand that the process of storage will cost us a storage fee which will be charged to us on an annual basis and on default of any payment whatsoever such default will constitute such embryo(s) as being regarded

as *"unclaimed embryo(s)"* as envisaged in terms of the Regulations to the Act and the authorised institution reserves the right to destroy the embryos.

17. If Genetic testing / PGS has been performed on our embryos we give permission to Vitalab to discard all the abnormal / aneuploid embryos.

18. We give Vitalab permission to utilise these abnormal / aneuploid embryos for training purposes in the laboratory before they are discarded.

Initials: _____ _____

19. 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.

20. We understand that medical aid coverage for any or all of the above procedures might not be available and that we are personally responsible for the expenses incurred during treatment. These expenses have been discussed with us by the competent persons.

21. We understand that we can choose to discontinue participation in the programme at any time, provided we have notified the authorised institution of our written consent thereto. We also understand that, should we decide to discontinue participation in the programme, we will be responsible for all expenses during the period prior to discontinuation and the relevant treatments incurred.

22. We understand that this consent extends from the start of the treatment programme until the treatment is completed, or until we decide to discontinue participation. This consent is binding, and required for participation in subsequent treatment programmes.

23. We understand that, should the results of our treatment and/or any aspect of it be published in medical or scientific journals, all possible precautions will be taken to protect our anonymity. We grant permission to the medical team to publish in professional journals relating to our case, provided our names are withheld.

24. In light of having selected either one or more of the processes listed at A – C on page 1 of this consent, we wish to commence with treatment:

24.1. We consent that all information supplied by us in connection with the removal and withdrawal of our gamete(s) be updated in the central data bank;

24.2. We have previously made a donation of our sperm / eggs.

Initials: _____ _____

24.2.1. If yes, please fill in the details below.

In the event that we have previously made a donation of our gamete(s), such donation took place on the _____ (date) at _____ (place).

Initials: _____ _____

24.3. We consent to a competent person of the authorised institution to perform:

24.3.1. A physical examination and a medical history questionnaire;

24.3.2. To remove or withdraw the gamete(s) for testing, analysis or any other process as such competent person of the authorised institution might deem necessary under the circumstances;

24.3.3. Provide our full particulars as per the terms of Regulation 8(1)(a)(ii), (iii) and (iv), (b), (c) and (f) of the Act.

24.4. We confirm that:

24.4.1. The recipient of us has undergone a prior gynaecological examination where applicable;

24.4.2. We will subject ourselves to a psychological evaluation by a competent person referred by the authorised institution.

24.4.3. I confirm and undertake that it is my responsibility to update my personal details in respect of contact details, and change of my status. This will allow the authorised institution and/or the competent persons of the authorised institution to contact me and to update their records to their satisfaction

25. The provisions of Regulation 18 of the Act related to the ownership of our sperm and/or our eggs, respectively, and the ultimate embryo(s) have been explained to us, and we undertake to read the provisions of section 18 which has been made available to us by the authorised institution.

26. We confirm that should the programme result in the successful birth of our child and/or a multiple births of our children, that it is our responsibility to notify the authorised institution and/or the competent persons of the authorised institution and disclose the following information:

26.1. Confirmation of birth(s);

26.2. The unique identification number which appears in our file.

26.3. Confirmation of any genetic disorders and/or birth defects in our child/children.

27. We agree that said notice, to the authorised institution, alternatively a competent person of the authorised institution, will be done within 30 days of the birth of said child/children.

28. We declare that the information which we have provided on this document, is indeed true and correct in every respect.

29. We confirm that we have read and understood this consent form. All of our questions have been answered to our satisfaction. All blanks were completed prior to our signing.

Signed at: _____

On the _____ day, of the_____ Month, of the year 20_____.

_____ _____

(SIGNATURE OF RECIPIENT) (SIGNATURE OF PARTNER)

_____ _____

(PRINTED NAME OF RECIPIENT) (PRINTED NAME OF PARTNER)

_____ _____

(SIGNATURE OF WITNESS) (SIGNATURE OF PERSON OBTAINING CONSENT)

_____ _____

(PRINTED NAME OF WITNESS) (PRINTED NAME OF PERSON OBTAINING CONSENT)

Appendix E: Ethics Clearance Certificate

R14/49 Dr Skye de Jongh et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170611

NAME: (Principal Investigator)	Dr Skye de Jongh et al
DEPARTMENT:	Obstetrics and Gynaecology Vitalab Centre for Assisted Reproduction
PROJECT TITLE:	GIFT and ZIFT: Is there still a Place for these Procedures in a New Era of Assisted Reproduction?
DATE CONSIDERED:	30/06/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Amy Wise
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	03/07/2017

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 Research Office Secretary in Room 301, Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in June and will therefore be due in the month of June each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 13/07/17

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix F: Journal Guidelines for Fertility & Sterility

FERTILITY AND STERILITY® MANUSCRIPT FORMAT

INTRODUCTORY PAGES

—Page 1: Running title not to exceed 40 characters (spaces and letters).

—Page 2: Title page (see Sample 1)

- —The title should be 3 lines or 150 characters maximum.
- —See Criteria for Authorship (Sample 2). Please note that there is a maximum of six authors.
- —Full names of authors and highest awarded academic degree. (Do not include honorary degrees.)
- —The author's(s') institutional affiliation and location at the time of the study should be included on the line beneath the author's(s') names. If the author(s) has moved since the study was completed, indicate in a footnote the "present address" for the appropriate author.

The following information, if applicable, should be included as footnoted items (beneath the institutional line of affiliation) to the title page. Order of footnote symbols: [a] [b] [c] [d] [e] [f]

- —Department affiliation of each author.
- —Present address of any author if different from institution where study was completed.

The following information should be included on the title page without a footnote symbol:

- —**Conflict of interest:** *On submission, the author(s) must disclose any potential conflicts of interest, whether of a financial or other nature. They must identify any financial arrangement they may have made with a company whose product is prominent in the submitted manuscript or with a company making a competing product. All sources of financial support must be listed on the title page. Commercial affiliations must be disclosed, regardless of whether they are a source of funding.*
- —Financial support (provide full name of funding institution; do not abbreviate); grant number, location (city, state, and/or country).
- —Include name of society, location, and dates if presented at medical meeting.
- —Provide name and address of author for reprint requests with fax number; e-mail should be included.

—Page 3: The capsule of abstract should be double spaced and should describe the *final conclusions* in 30 words or less.

> In a prospective, randomized study, no significant advantage was found in the precise timing of hCG administration after pituitary desensitization before ovarian stimulation for IVF.

—Page 4: Structured abstract (see Sample 3)

- —Length: 175 to 200 words (maximum); double-space. (Omit *P* values.)
- —A structured abstract now is required for all articles, including Modern Trends, Case Reports, and Techniques and Instrumentation.
- —Key words. Provide 3–10 key words or short phrases below the abstract that will assist indexers.

MANUSCRIPT FORMAT

—Limit of 1 figure or table for every 3 typewritten pages *of text* or approximately every 600 words.

—Manuscript includes main headings: **Introduction, Materials and Methods, Results,** and **Discussion.** (See Sample 4 for headings format.)

—All abbreviations, including Universal Abbreviations, must be written out in the title, abstract, and text where first cited, and at the beginning of a sentence. Abbreviations used in tables, figures, and legends must agree with text. The journal recognizes only internationally approved and accepted units of measure and abbreviations as noted in the "AMA Manual of Style" or "Dictionary of Medical Acronyms and Abbreviations" (2nd edition, edited by Jablonski). (See Sample 5 for Universal Abbreviations that must be used without definition.)

—Generic names must be used for all drugs. Use the trade name only if it is necessary to differentiate among drug forms or if a specific trade preparation was used. If a commercial product is cited, the name and location (city, state, and/or country) of the manufacturer must be included in parentheses.

—Identify patients by number and *not* by initials.

—*Please submit a 3.5" floppy disk to the editorial office with your final revision (you do not need to submit a disk with your original manuscript).*

REFERENCES (see Information for Authors for samples)

—Begin references on a separate page and identify in the text by arabic numerals in parentheses in numerical sequence on line of text. Type double-spaced. References

appearing for the first time in tables and figures must be numbered in sequence with those cited in the text where
F1 the figure or table is first mentioned.

—References must be verified from the original sources and be in proper format. See ''Information for Authors'' with regard to style and reference limitations. Journal titles must conform to those used in Index Medicus.

—List all authors. If the number of authors exceeds six, give six, followed by *et al*.

—If author's(s') own papers are cited as ''in press'' in the reference list, two copies of such papers *must* be provided. The title of the journal and expected month/year of publication must also be included. Unpublished data and personal communications may *not* be used as references, although references to written, not oral, communications may be inserted (in parentheses) in the text (e.g., J. Colton, personal communication).

—Please avoid citing abstracts as references. Abstracts can be cited as references only if they have been published

TABLES (See Sample 6)

—Tables must be typed double-spaced on separate pages, titled, and numbered with arabic numerals.

—Footnotes must be identified using the following symbols:
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—Omit internal horizontal and vertical lines.

—Data in tables cannot be duplicated in text or in figures.

ILLUSTRATIONS

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—See below for an example of a figure prepared according to the journal format.

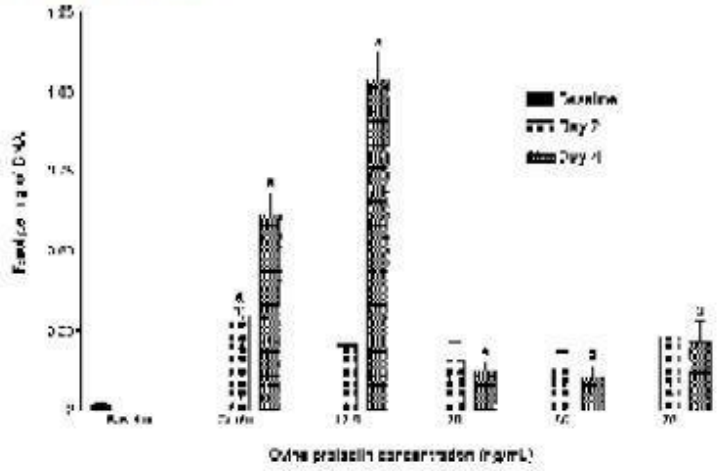
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—The manuscript (text, references, tables, and legends) must be typed double-spaced on white paper (216 × 279 mm [8 1/2 × 11 in]) with margins of at least 25 mm (1 in). Submit an original and 2 copies. Include the page number on each page.

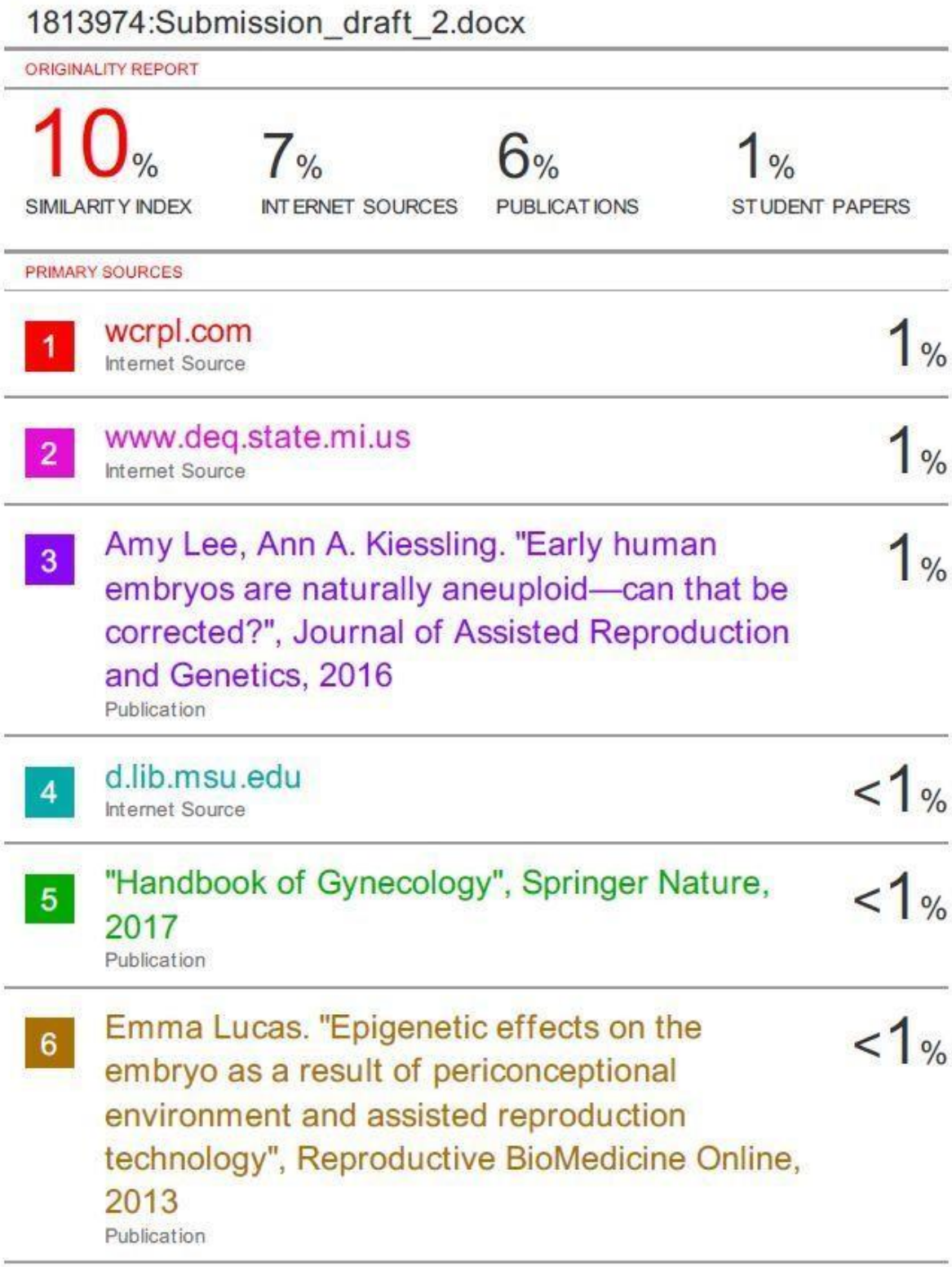
—Conventional units must be used for linear dimensions and hematologic and clinical-chemistry measurements. Use the degree Celsius for temperatures.

—Recognition of gifts or donations should be included in an acknowledgment section, not in the text. Individuals identified in the acknowledgment should have full names included, degree(s) if applicable, institutional affiliation and location (no abbreviations). Authors are responsible for obtaining written permission from persons acknowledged by name because readers may infer endorsement of the data and conclusions.

EXAMPLE OF FIGURE



Appendix G: Turnitin plagiarism report



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Appendix H: Plagiarism declaration

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FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Skye de Jonah (Student number: 1813974) am a student registered for the degree of MMed Obstetrics + Gynaecology in the academic year 2017.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

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