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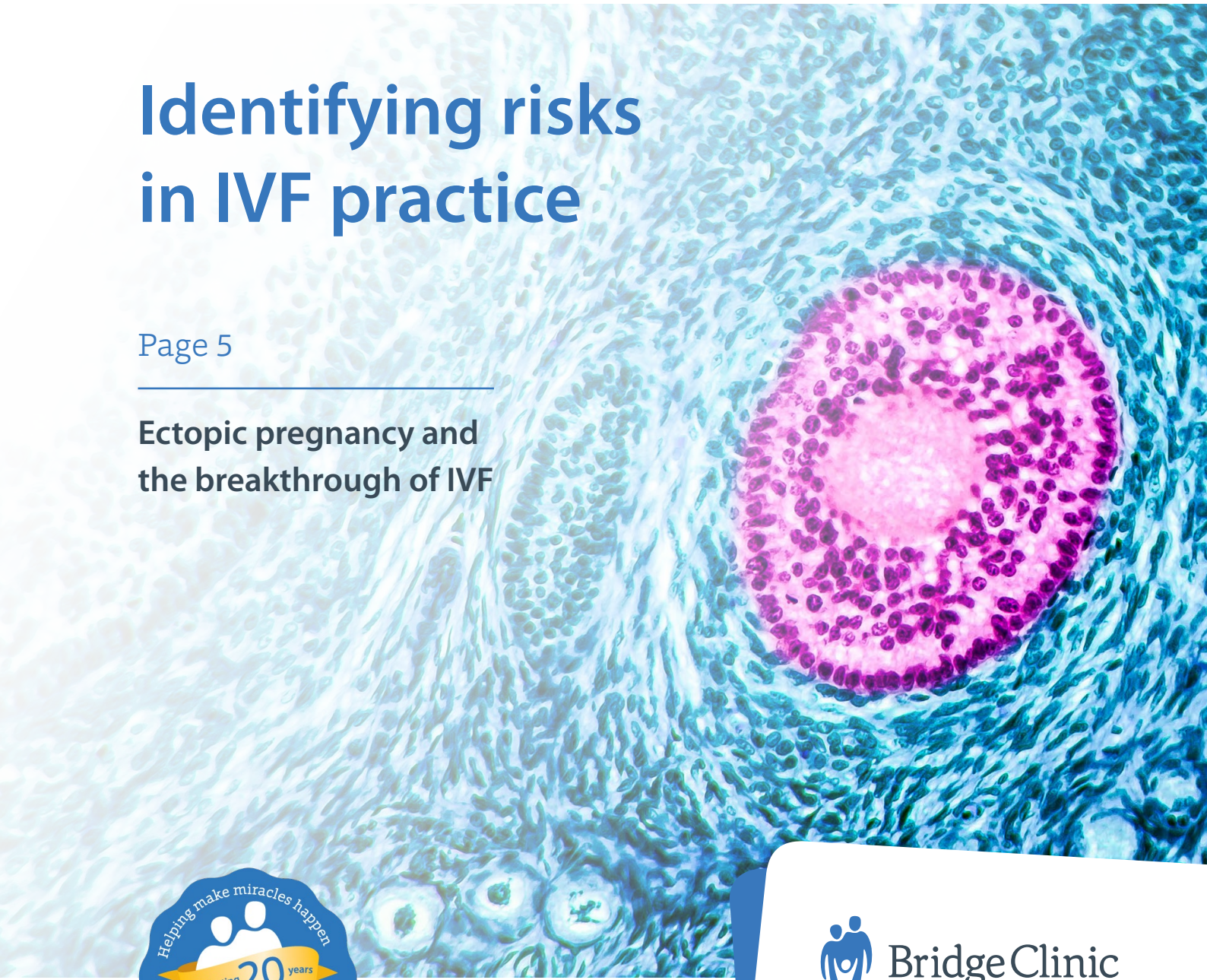


Identifying risks in IVF practice

Helping make miracles happen
Celebrating 20 years
Bridge Clinic
From 1999 - 2019

Bridge Clinic

www.thebridgeclinic.com

20 Years of bringing joy into this world

The only impossible journey is the one you never begin…

Bridge Clinic was birthed by a Nigerian doctor practising in the UK, who had a dream of one day being a part of developing the healthcare sector in Nigeria to match the best in the world. Founded by Dr. Richardson Ajayi, Bridge Clinic gradually transformed from a sole proprietorship to a world class, functionally independent entity, putting smiles on the faces of now more than 2,000 families.

"I was surprised by the large number of Nigerians that were constantly coming to the UK for treatment. After a few years of watching them, my entrepreneurial nerve started to twitch and I thought to myself, there's a major opportunity in Nigeria."

– Dr. Richardson Ajayi

As a young consultant gynaecologist, Dr. Ajayi started Bridge Clinic to offer IVF services to couples facing infertility challenges. Prior to the establishment of Bridge Clinic, Nigerians who needed IVF treatment and who could afford it had to travel out of the country to access care. Middle-income households who could not afford such a luxury where left with little or no credible alternatives. Bridge Clinic's vision has always been to deliver world-class healthcare in Nigeria to as many people as possible. It was from this vision that Bridge Clinic was created and to give Nigerians the option to have IVF treatment that rivals and surpasses international benchmarks.

As with any pioneering initiative, establishing a quality focused IVF clinic back in the middle 1990's came with a number of challenges and setbacks.

Irrespective of the difficulties, Bridge Clinic aligned with the mantra, "The only impossible journey is the one you never begin." In 1996, the company was incorporated and set up with technical support from the assisted conception unit of Kings College Hospital in London. By 1998, the Embryology lab was commissioned and the clinic became operational.

IVF was a largely unknown service in Nigeria at the time and was considered by most as too strange or experimental. Reservations on the normality of children that were born from IVF intervention, were largely responsible for the slow uptake. It took a lot of persuading and convincing for the first clients to agree to treatment.

In 1999, less than a year after opening, Bridge Clinic's first IVF babies were born, a set of twins. Though live births were recorded, results during the early years were below global average standards. An analysis into the poor results suggested that success rates could be improved on with the introduction of a more advanced, and at the time, experimental procedure known as Intracytoplasmic Sperm Injection (ICSI).

Infertility treatment can be a complex matter and though Intracytoplasmic Sperm Injection (ICSI) was introduced it was observed that other specialist care was needed to ensure continued improvement of success rates. Never one to shy away from a challenge and adopting new technology and procedures, Bridge Clinic became the first clinic in Nigeria in 2000 to deliver a baby born as a result of Intra Cytoplasmic Sperm Injection (ICSI) and surgical sperm collection.

The lessons during the early years became the foundation stone and pillars upon which the clinic was built. It necessitated the need to always act in an ethical manner, the need to be innovative and to adopt the latest in medical advancement, and the need to focus on excellent service delivery through a quality management system.

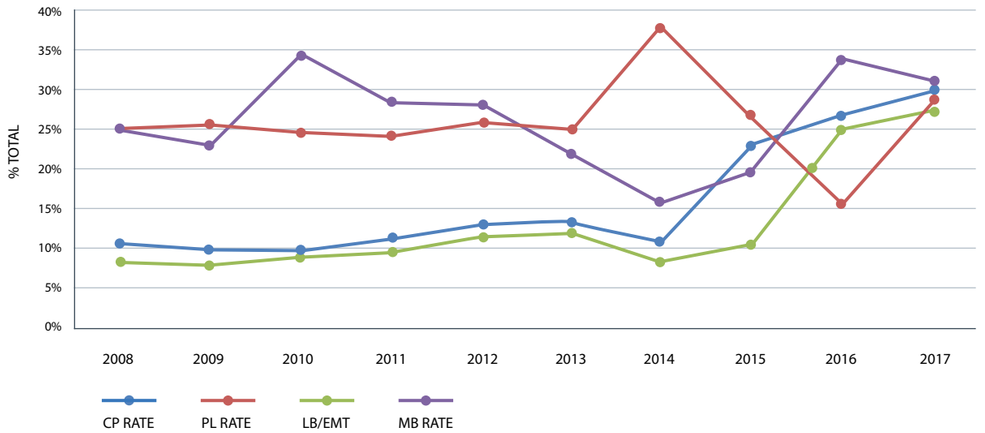
From the onset, the goal was to practice IVF within the sustainable context of a quality management system ensuring consistent service delivery and putting patient safety first. In the same year, Bridge Clinic inaugurated an ethics committee to codify a values-based treatment protocol. As an organisation, Bridge Clinic believes in putting strong values first and as a result of the ethics committee, never compromised on these values. The organisation willfully pass up treatment opportunities and the attendant revenues if client cases are not agreeable to the values of the clinic. One such policy is to not transfer more that two (2) embryos into a client during any treatment cycle. This policy is in place as the patient safety risk is deemed too high, and contravening this policy can put the lives of the mother and the unborn child at risk.

Doing business in Nigeria is fraught with challenges. Bridge Clinic's experience is no exception and during the past 20 years, the clinic had to devise ingenious solutions to resolve issues relating to continuous water availability, uninterrupted power supply, importing and setting up of equipment while at the same time ensuring effective maintenance of equipment and operational standards.

One lesson that Bridge Clinic has imbibed as a business is the attitude of continuous improvement. Figure 1.0 below shows the progress Bridge Clinic has made with regards to pregnancy rate and live births over the past ten (10) years. Honesty and accountability are fundamental values of the clinic and as such, all results that are published are first audited by international consulting firm, Alexander Forbes.

Bridge Clinic Treatment Records Validation as at December 2017

Fig. 1.0 Time Plot on Birth Rates



As an organisation, Bridge Clinic has continuously delivered superior results to our clients. To accelerate the growth process and our learning curve, Bridge Clinic entered into a technical partnership with IVF centres Prof. Zech in 2014. IVF Centres Prof. Zech is a leading IVF clinic and research laboratory based in Austria and with operations across 6 European countries. The result of this partnership significantly improved Bridge Clinic's results in 2015 and we have been able to sustain this improvement continuously since then.

Bridge Clinic, in partnership with its strong international collaborations, is passionately committed to help individuals with fertility and general health issues, in spite of the ominous healthcare situation in our society. We remain committed to deliver world-class healthcare to everyone.

Our reputation to never compromise on quality has established trust with our patients. This trust has resulted in patients requesting everyday health services and in 2017, Bridge Clinic opened its first of currently three medical centres that focuses on a broad range of general medical services for everyday health concerns. To mark our 20 year anniversary, we are happy to announce our return to Victoria Island later this year.

We thank each and every one of our doctors and partners who have placed their trust in our ability to deliver on quality services and who have helped us play our part to transform the healthcare sector in Nigeria thus far. Together, we can do more.

Trust is earned

Bridge Clinic: Investing in today and the future

The leading fertility clinic in West Africa operates under internationally monitored accreditation with a quality management system that is certified and regulated by Quality Austria. Teams are highly qualified, professional and experts in their field, giving couples their best chance of conceiving.

By recruiting only the best personnel and developing both staff and facilities through ongoing training and investment in equipment, Bridge Clinic Fertility Centre has grown from strength to strength over the past 20 years.

Read more at www.thebridgeclinic.com.

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Contents

Fertility preservation and the 21st century nurse

By Sekinat Kayode-Lawal, Fertility Nurse - Bridge Clinic Fertility Centre, Lagos

Definition

Fertility preservation is the process of saving or protecting eggs, sperm, or reproductive tissue to be used to have biological children in the future.

It is important that registered nurses understand the reality of what is possible now, what may be available in the future, and to support people to manage fertility should they become infertile due to illness/treatment or through choice for non-medical (social) reasons.

Who can benefit from fertility preservation?

People with certain diseases, disorders, and life events that affect fertility may benefit from fertility preservation. These include people who:

- Have been exposed to toxic chemicals in the workplace or during military duty
- Have endometriosis
- Have uterine fibroids
- Are about to undergo chemotherapy
- Are about to be treated for an autoimmune disease, such as lupus
- Have a genetic disease that affects future fertility
- Social freezing (desire to delay childbearing in order to pursue educational, career or other personal goals)

What are the fertility preservation options?

Male options

1. Sperm cryopreservation: This process involves freezing semen. Sperms are washed, spermatozoa concentrate are frozen in liquid nitrogen and stored for future use. This can be carried out from around 13 (around puberty). Legally, sperm can be stored for a person for up to 55 years or on a ten-year rolling basis. And once ready, the sperm in a semen sample can be used to fertilize the eggs recovered in an IVF cycle using intracytoplasmic sperm injection.

Semen samples can be discarded if reproductive function is restored/established after successful treatment, or they can be donated for research purposes.

2. Testicular tissue freezing: Testicular tissue freezing is a technique to preserving the fertility of men who do not produce viable sperm in their ejaculate (azoospermia).

Female options

1. Embryo cryopreservation: This method, also called embryo freezing, is the most common and successful option for preserving a female's fertility. First, a health care provider removes eggs from the ovaries. The eggs are then fertilized with sperm from her partner or a donor in a lab in a process called in vitro fertilization. The resulting embryos are frozen and stored for future use.

2. Ovum freezing or cryopreservation: This is when unfertilized eggs are 'vitrified' / "frozen" and stored. There have been successful cases of oocyte vitrification in adolescent females.

3. Ovarian transposition: A health care provider performs a minor surgery to move the ovaries and sometimes the fallopian tubes from the area that will receive radiation to an area that will not receive radiation. For example, they may be relocated to an area of the abdominal wall that will not receive radiation. This is used in cancer patients.

4. Ovarian tissue freezing: Research has shown that freezing ovarian tissue can be an effective method of allowing women to have a successful pregnancy after cancer treatment if they don't have time to freeze their eggs. It is also the only option to preserve the fertility of younger girls who have not started ovulating.

Role of the nurses

An informed nurse is a go-to information resource for clients who have these desires. She counsels on the possibility of future infertility with patients of reproductive age or with parents/guardians of children as part of the education. This also includes talking them through the consent process before treatments are initiated. Nurses also play a major role in counseling the client before commencement, during and after treatment course.

Furthermore, she does the following;

- Is always prepared to have the initial discussion, be familiar with the potential for fertility preservation and understand the importance of early referral to fertility specialists.
- Is informed with all fertility preservation options (local and national) and is familiar with local referral processes to reproductive specialists.
- Enlightens the clients on potential side effects, complications that could arise from the process of preservation.
- Performs an informative role to clients on the legal and regulatory requirements, as well as the ethical, cultural and religious considerations, associated with fertility preservation and treatment.
- Should also engage clients on how to legally give their consent to treatment, and also allows them decide what could happen in case of any eventualities.
- Understands and supports the client through the emotional issues and requirement for sperm/egg collection at a time of crisis.

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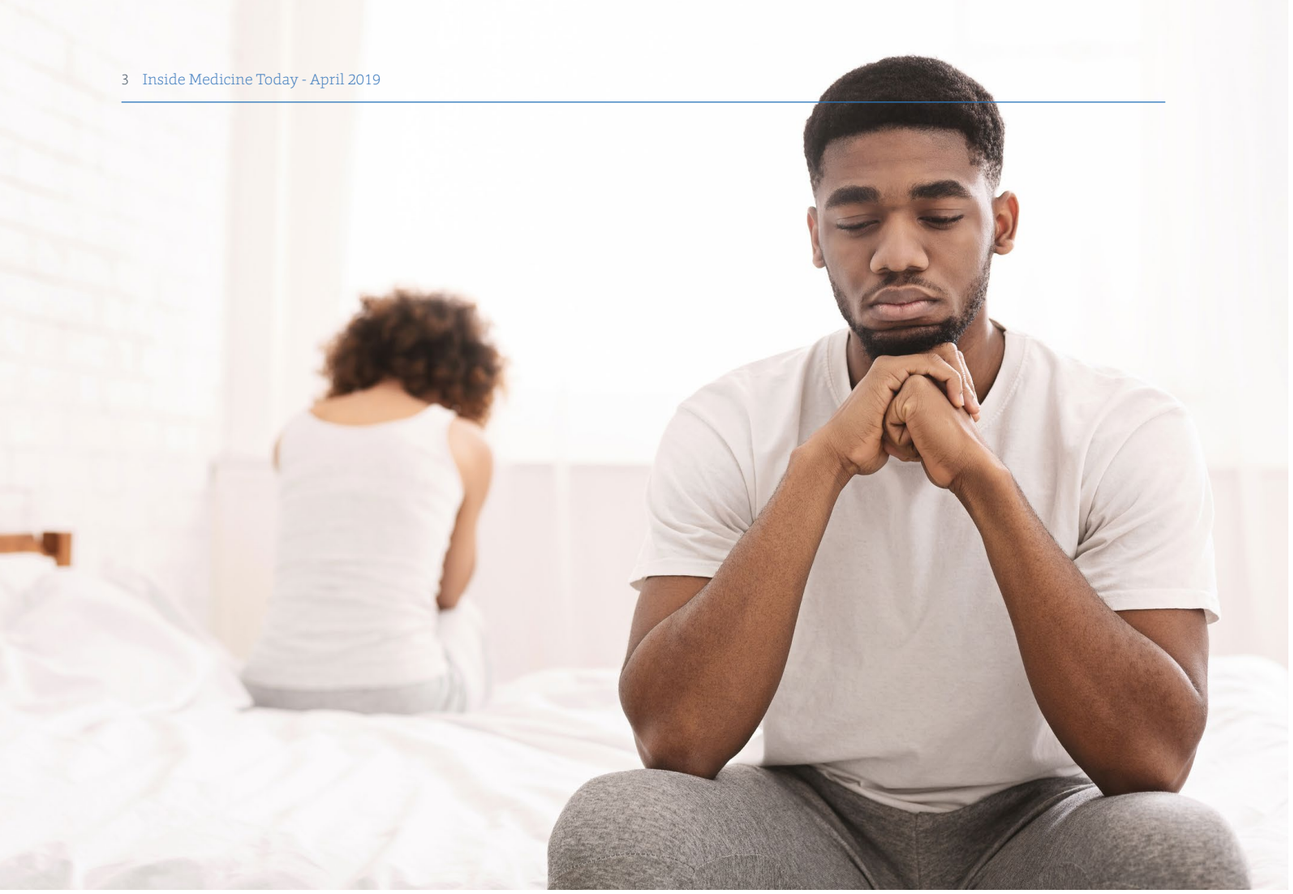

Overcoming the jitters: A vital step in conquering infertility

By Joseph Olayinka Uwa, Clinical Embryologist - Bridge Clinic Fertility Centre, Lagos

Infertility affects an estimated 10-15% of couples of reproductive age. Studies suggest that after 1 year of having unprotected sex, 12% to 15% of couples are unable to conceive, and after 2 years, 10% of couples still have not had a live-born baby.

In couples younger than age 30 who are generally healthy, 40% to 60% are able to conceive in the first 3 months of trying.[11]

Keywords

anxiety | depression | distress | IVF | infertility | psychosocial support | quality of life

Several studies have indicated that infertile couples experience high levels of stress, depression, and anxiety. Emily et al., 2018 suggested that multiple risk factors for anxiety and depression during infertility treatment have been identified; these include being female, age over 30, lower level of education, lack of occupational activity, a male cause for infertility, and infertility for 3-6 years. In addition, studies have indicated that higher levels of depression and anxiety are associated with lower pregnancy rates among patients undergoing IVF.[5]

Patients who are struggling to conceive report feelings of depression, anxiety, isolation, and loss of control.[9] It is estimated that 1 in 8 couples (or 12% of married women) have trouble getting pregnant or sustaining a pregnancy.[4] Despite the prevalence of infertility, majority of infertile women do not share their story with family or friends, thus increasing their psychological vulnerability. The inability to reproduce naturally can cause feelings of shame, guilt, and low self-esteem. These negative feelings may lead to varying degrees of depression, anxiety, distress, and a poor quality of life.

Why does anxiety affect fertility?

Currently it is unclear why anxiety seems to have an effect on female fertility. It is also possible that anxiety and stress alter hormones and make it much harder for the body to effectively function. We know that stress can cause women to stop having periods, and stress can lower sperm count in men. Perhaps hormones and the way they are altered by anxiety play a significant role. No matter the cause, it is clear that anxiety is problematic especially for female who constantly undergo stress and pressure.

Reducing anxiety and improving the chances of conception

There is no magic formula for improving anxiety and fertility. Unfortunately, if you continue to put pressure on yourself, or you still deal with profound anxiety in the rest of your life, you may find that it affects your overall fertility levels.

But there are some strategies that can be helpful. These include:

I. Seeing a Doctor: Make sure that you are at least seeing a doctor. The truth is that as much as people want to avoid finding out that they're infertile, the anxiety of thinking that you are can be just as problematic, if not more so. It is best to see a doctor, find out if everything is okay, and then do what the doctor recommends.

II. Relaxation Months: While you can always plan to continue to try to conceive, try taking a one month on, one month off approach to conception. Consider the month off a chance to relax, where you're not watching a calendar and you go in with no expectations. Obviously this can be easier said than done, so consider other relaxation strategies like massage, yoga, etc. so that your "months off" are as relaxing as possible. Consider adding exercise as well, which is known to reduce anxiety - but moderately since it may have an effect on fertility in extreme cases.

III. Making Sex Fun: It is also important that conception never feels like a chore. That means that you should have fun with it. You and your partner should find trying to conceive something you look forward to even if conception does not occur. That will reduce some of the stress of the entire situation. Anxiety can cause a range of side effects that can make it more difficult to conceive. When you are anxious or depressed it is common to have a lower sex drive, and to feel more tired, for example.[10] This can lead to less frequent sex, making it harder to fall pregnant.

In summary, rates of anxiety and depression are high among couples undergoing infertility treatment. Several studies have demonstrated that these symptoms may diminish the chances of conception. Both medication and cognitive behavioural therapy can be helpful in alleviating these symptoms. In Bridge Clinic, all clients are availed the opportunity to get proper counselling from the care and support team and to seek a referral if they are experiencing symptoms of depression or anxiety during the course of treatment.

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Ectopic pregnancy and the breakthrough of IVF

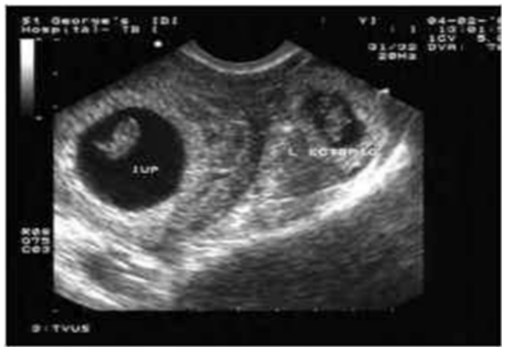
By Oluwagbemisola Aremu, RN: RM: Fertility Nurse - Bridge Clinic Fertility Centre, Lagos

Ectopic pregnancy is any pregnancy with the fertilized embryo implanted on any tissue other than the uterine lining.

Ectopic pregnancy (EP) is the leading cause of maternal morbidity and mortality during the first trimester and the incidence increases dramatically with in vitro fertilisation and embryo transfer (IVF-ET).

The presence of simultaneous pregnancies in two different implantation sites, generally one intrauterine and one extra uterine is simply Heterotrophic Pregnancy.[11]

The co-existence of an EP with a viable intrauterine pregnancy (IUP) is known as heterotopic pregnancy (HP) affecting about 1% of patients during assisted conception.



Prevalence

EP is estimated to be 1-2% of all natural conceptions and the incidence increases following ART. It causes major maternal morbidity and mortality with pregnancy loss and its incidence increasing world wide.(Storeide o et al 1976). It can reach up to 11% in female patients with a history of tubal factor infertility. The incidence of EP with IVF/ET has been reported to be between 2.0% and 3.5%.[9]

Pathophysiology

The explanation for EP during IVF-ET would be impairment of tubal function and endometrial receptivity with ectopic implantation occurring following failure of the normal biological interactions between endometrium, Fallopian tube and embryo due to controlled ovarian stimulation and the subsequent alteration in hormonal milieu.

Hence women with underlying tubal disease undertaking IVF may face a "double whammy" in risk of EP, due both to the tubal disease and adverse effects of superovulation on tubal function during the IVF cycle.

Risk factors

- Maternal Factor/Pathophysiological
- Embryo Factor/Physiological
- Transfer Factor/Mechanical

Maternal factors

- Tubal factor infertility: compared with other causes of infertility, increases the risk of EP following IVF-ET with a prevalence of up to 11%.[6]
- Pelvic Inflammatory Disease
- Endometriosis
- Uterine factors

Embryonic factors

- Quality of embryo: Higher grade embryos have the tendency to migrate and implant in the wrong site
- Assisted hatching: This allows the embryo to implant much more easily at any comfortable position for it

Transfer factors

- Technique of transfer: This could also influence the rate of ectopic implantation due to forcing the embryo through tubal ostia by hydrostatic pressure or by using large volume of transfer medium.
- The position of transfer catheter and the distance from the fundal endometrium to either the tip of catheter or to an air bubble within the catheter have also been investigated as potential risk factors for low pregnancy rate and development of EP.
- Full bladder at transfer

Signs and symptoms

The signs and symptoms of an ectopic pregnancy typically occur six to eight weeks after the last normal menstrual period.

- Abdominal pain
- Vaginal bleeding
- Weakness
- Dizziness and a sense of passing out upon standing (also termed near-syncope) can be signs of serious internal bleeding and low blood pressure from a ruptured ectopic pregnancy and require immediate medical attention.

How to diagnose it

Ultrasonography can definitely diagnose an ectopic pregnancy when a gestational sac with a yolk sac or embryo, or both, is noted in the adnexia (Barnhart etc all.)

- By 5 to 6 weeks of pregnancy (1-2 weeks after ET) all normal pregnancies in the uterus should be seen by vaginal ultrasound
- With increasing rise of BHCG without a gestational sac seen in the endometrial, there is a 70% chance of ectopic gestational
- The usual ultrasound finding for an ectopic is a mass on one side, some fluid in the pelvis, and no normal pregnancy structures in the uterus.
- In about 20% of ectopics with vaginal ultrasound, a fetus or fetal cardiac motion is seen outside the uterus.
- Conclusive diagnosis of ectopic by ultrasound can only be made if a fetus or fetal cardiac motion is seen outside the uterus. This is only seen in about 20% of ectopics with vaginal ultrasound
- A "pseudo sac" is seen in 10-20% of ectopics. This is a sac in the uterus that is not a pregnancy but can look like one very early on. We need to see a yolk sac, fetal pole or cardiac motion to know it is a gestational sac
- Laparoscopy: This is the gold standard diagnosis. When there is a high index of suspicion, as well as ultrasound findings suggestive of an ectopic and/or suspicious beta HCG levels and/or symptoms, a laparoscopy should be done.

HCG Levels in early pregnancy

Number of weeks	Levels of HCG (IU/ml)
3 weeks LMP	5 - 50
4 weeks LMP	5 - 426
5 weeks LMP	18 - 7,340
6 weeks LMP	1,080 - 56,500
7 - 8 weeks LMP	7,650 - 229,000
9 - 12 weeks LMP	25,700 - 288,000
13 - 16 weeks LMP	13,300 - 254,000
17 - 24 weeks LMP	4,060 - 165,4000
25 - 40 weeks LMP	3,640 - 117,000

Medical management

Methotrexate

Systemic methotrexate has been used to treat gestational trophoblastic disease since 1956 and was first used to treat ectopic pregnancy in 1982.[12]

Methotrexate inhibits rapidly growing cells such as a pregnancy or some cancer cells.
The criteria for Methotrexate are as follows;

- Hemodynamically stable
- No evidence of tubal rupture or significant intra-abdominal haemorrhage
- No fetal cardiac activity
- Mass in tube is less than 3-4 cm diameter
- No contraindications to Methotrexate
- Informed consent from the patient
- Willingness to attend for follow up

Surgical management

Laparoscopy is the method of choice in most patients. Cases of rupture with significant bleeding into the abdomen are usually done by laparotomy since it can be done faster

- Salpingotomy (or -ostomy): Making an incision on the tube and removing the pregnancy
- Salpingectomy: Cutting the tube out
- Segmental Resection: Cutting out the affected portion of the tube
- Fimbrial Expression: "Milking" the pregnancy out the end of the tube

In general, the procedure of choice is Salpingectomy unless there are fertility-reducing factors present.

Studies about ectopic pregnancy and IVF

Elsevier 2015

43 of 48 EP occurred in patients with tubal factor infertility.

Pathological findings revealed tubal lesions in all 46 Salpingectomies.

Ectopic pregnancy after IVF appears related to pre-existing tubal pathology.

Eshre 2015

A nationwide analysis of all ART pregnancies achieved in UK between 2000 and 2012 following all 161,967 IVF and ICSI

The result showed that 1.4% of all pregnancies were ectopic

When the ectopic examined, the major risk factor was the presence of a tubal pathology which doubled the risk

Taiwan 2015

A retrospective study of pregnancy achieved after IVF at O&G DEPT Kaohsiung Chang gung Memorial Hospital between 1999 and 2013.

Results showed that out of 1213 pregnancies,18 were ectopic

TET and ET under full bladder distension had a significant effect on ectopic rate

Bridge Clinic 2017

A study of all 4EP between June 2015 - June 2017 revealed

2 had tubal factor infertility

3 had cervical adhesions

3 had very difficult transfers

3 had pseudo sacs on scan

Good practices that reduce ectopic rate

- Removal of hydro salpinges
- Use of right catheter
- Not touching the fundus
- Avoiding the use of a tenaculum/vulsellum
- Ultrasound details of uterine cavity before transfer
- Keeping catheter stationary for at least one minute
- Accurate Dummy transfer before treatment
- Ultrasound-guided transfer

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Innovative approaches to waiting time management in healthcare

By Anne Nmemah, Clinic Administrator - Bridge Clinic Fertility Centre, Lagos &
Ukemeobong Owoh, Senior Project Officer - Bridge Clinic

Abstract

The purpose of this paper is to compare the impact of various time management approaches and measure their effect on the reduction of waiting times with the view to delivering patient-centric care at all stages of IVF treatment. Waiting time is a persistent problem in healthcare. This is even more so in a system where appointments are pre-scheduled for clients.

A late client, or even an overly early client can trigger negative domino effects and lead to a waiting time build-up within the system.[1] This increases stress and reduces patient satisfaction. The negative effects of waiting time are unwanted, especially given that IVF is already an emotional nightmare for many clients – even in the best of environments.[2] Our approach to solving the waiting time issue focuses on three independent approaches layered on each other over a three month period. Our first approach was to deploy a floor manager to manage the flow of resources.

The second approach – layered on the first – involved retraining clinical staff on pre-defined standard operating procedures, and the third approach involved developing new work schedules to optimize client flow through the system. The results of our approach showed an 88% reduction in waiting time.

> "SOP refresher meetings helped reinforce core processes and eliminate any non-value adding activities"

Introduction and motivation

This study shows the positive impact that simple changes in clinical team management and administration can have on the quality of patient-centered care – within the context of waiting time.

Methodology

Our approach consisted of a baseline determination and four experiments.

Baselining

To accurately measure the impact of our work, we began by running a baseline assessment across a panel of eight (8) selected procedures: appointments, Standard Investigation Review (SIR), scans, Ultrasound Directed Follicular Aspiration (UDFA), Embryo Transfer (ET), Frozen Embryo Transfer (FET), Surgical Sperm Collection (SSC).

Experiment 1

Floating Floor Manager

The first experiment was the assignment of a floor manager dedicated to monitoring waiting time. The floor manager role is a floating role based on a schedule filled by nurses and front-of-house (receptionists). The role of the floor manager is to continuously manage patient waiting time experience for the entire work-day.

The experiment with a floor manager continued for a two-week period. This allowed various people to rotate in and out of the role – thus correcting for variances in personality traits, administrative competence and differences in client volumes on different days of the week.

Experiment 2

Standard Operating Procedure (SOP) Re-Training

Our second experiment involved training clinical staff on our client management Standard Operating Procedures (SOP). These SOP trainings occurred during clinical meetings and during daily clinical meetings. These SOP refresher meetings reinforced key processes across core processes and eliminated any non-value adding activities introduced based on personal experience and/or client pressures.

The balancing measures for this experiment were measuring compliance to SOPs through compliance audits and spot checks.

Experiment 3

New Work Schedule

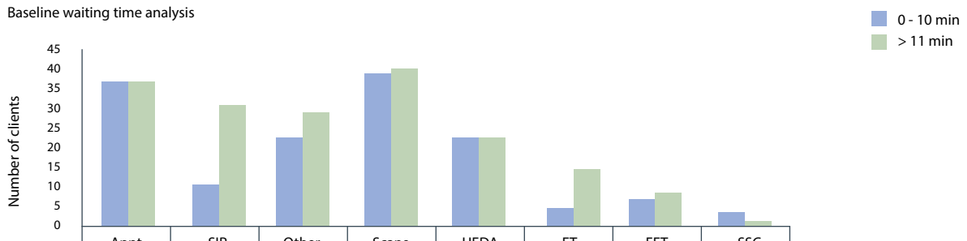
Our third experiment was to introduce a new work schedule to equitably redistribute work based on competence, experience and the daily work schedules.

The balancing measures for this experiment involved analyzing individual performance on a daily basis and providing feedback to each member of the clinical team to ensure accountability was clearly communicated.

Results

Baseline Assessment Results

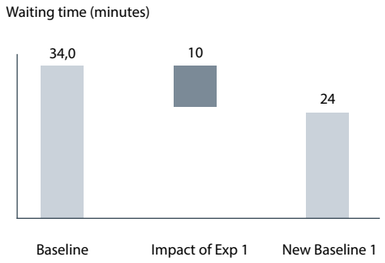
Within Bridge Clinic, our patient-centric metric for client satisfaction is a waiting time of less than or equal to ten (10) minutes. Out of a panel of eight (8) procedures under study, only one procedure was carried out under ten (10) minutes.



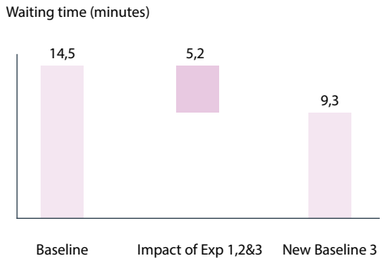
	Appt.	SIR	Other	Scans	UFDA	ET	FET	SSC
0 - 10 min	37	10	22	38	22	4	7	3
> 11 min	37	31	29	40	22	14	8	1

Average waiting time at the beginning of this study was 34 minutes

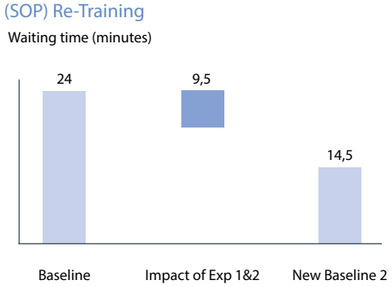
Experiment 1 Results – Floating Floor Manager



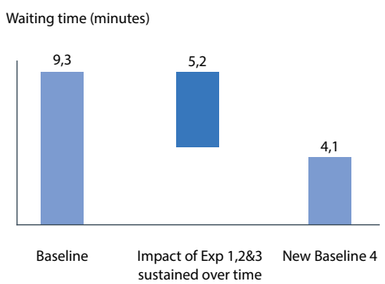
Experiment 3 Results – New Work Schedule



Experiment 2 Results – Standard Operating Procedure (SOP) Re-Training



All Experiments Sustained Over a Time Period



Discussion

Waiting time, in and of itself, may not be perceived as advantageous to clients when controlled for other clients. So, we tracked customer satisfaction as a patient-centered index to track the impact on clients. Over one period of these experiments, customer satisfaction index grew from 82% to 89% a 7-% point increase in waiting time.

Next Steps

To further enrich the literature, there is the need to express the time units in this study and dimensionless time invariants to account for volumetric changes. This will form the core of the next phase of the research work.

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PGT

The first miracle was "Yes, we do PGT" I'm looking forward to having my baby! Another baby!

"I bumped into my old school mate early 2012 and he advertised Bridge Clinic, and I said I wouldn't need their services because I was already pregnant with my first child. We had him (first baby, Nov 2012) and discovered he was HBSS. Wow! Then the fear of having another HBSS child delayed us for another 6 years. At this point I remembered my old school mate and couldn't get his contact, so we decided to come directly to Bridge Clinic. The first miracle was "Yes, we do PGT" i.e. to screen for genetic abnormalities. I started my treatment July, 2018 and did the transfer December same year. Fasted and prayed hard! Yes very hard!

I'd just say God had Mercy on me and I'm looking forward to having my baby! Another baby!

PRAISE THE NAME OF THE LORD!

And to the Bridge team, Dr Shittu, Dr Olaniyi, Nurse Kate, Gbemi and all of you who I didn't mention your names, God bless you real good and thank you for every encouragement. Front desk, thank you for always smiling, even when I'm late. God bless you."

- Mrs D.A.O

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"Sexual function is one of the important components of health and overall quality of life"

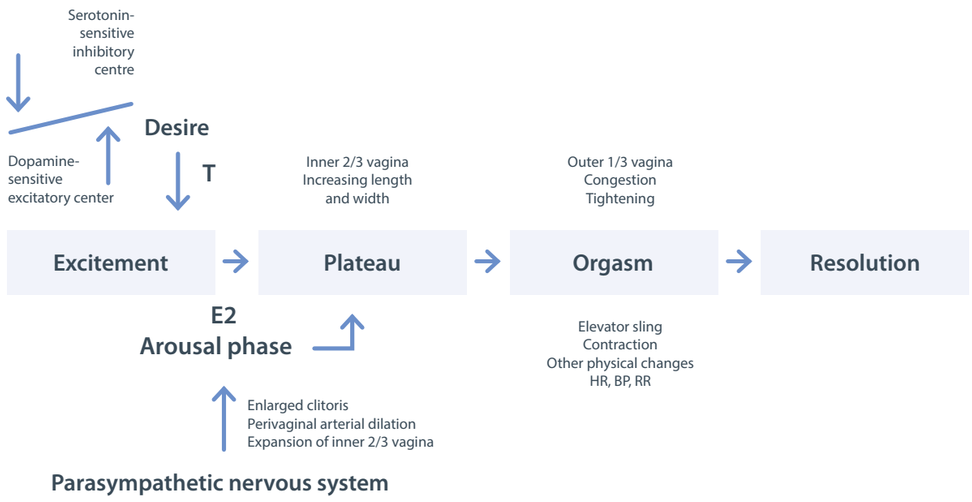
When sex becomes a chore: causes and remedies

By Ijeoma Uhiara, RN. RM. BSN. Fertility Nurse - Bridge Clinic Fertility Centre, Abuja &

Ann Odokara, RN. RM. BSN. Fertility Nurse - Bridge Clinic Fertility Centre, Abuja

Summary

Sexual dysfunction occurs when an individual/couple experience difficulty during any stage of a normal sexual activity.[1] This in turn results in lack of satisfaction at any of the four stages of the sexual cycle: excitement, plateau, orgasm and resolution. As a fall out on this, conception may become difficult for the couple. It can be diagnosed when an individual/couple feel extreme distress and interpersonal strain for a minimum of 6 months (excluding substance or medication-induced sexual dysfunction).[1]



Sexual dysfunction

Sexual dysfunction has been classified into four main categories

Desire Disorders	Arousal Disorders	Orgasm Disorders	Pain Disorders
Lack of interest in sex Caused by: • Hormonal factors • Psychological factors • Physical factors • Medications	**Lack of excitement during sexual activity** Caused by: • Erectile dysfunction • Premature ejaculation • Lack or absence of vaginal lubrication	**Anorgasmia** Caused by: • Physical • Psychological • Pharmacological	**Affects mostly women** Caused by: • Vaginismus • Dyspareunia • Vulvodunia

https://www.researchgate.net/figure/Physiological changes in the current model of the female sexual response cycle BP-14_fig1_236099314

Sexual dysfunction

- ***Desire disorders*** — lack of sexual desire or interest in sex
- ***Arousal disorders*** — inability to become physically aroused or excited during sexual activity
- ***Orgasm disorders*** — delay or absence of orgasm (climax)
- ***Pain disorders*** — pain during intercourse

Causes of sexual dysfunction in men and women

Physical causes: Many physical and medical conditions can cause problems with sexual function. These conditions include diabetes, heart and vascular (blood vessel) disease, neurological disorders, hormonal disorders, menopause, chronic diseases such as kidney or liver failure, and alcoholism and drug abuse. In addition, the side effects of certain medications, fatigue, can affect sexual desire and function.[7]

Psychological causes: These include work-related stress and anxiety, concern about sexual performance, marital or relationship problems, depression, feelings of guilt, and the effects of a past sexual trauma.[7]

Types of sexual dysfunction

- Orgasmic dysfunction
- Ejaculatory dysfunction: Premature ejaculation; ejaculation that occurs before or soon after penetration, Inhibited or retarded ejaculation; ejaculation is slow to occur, Retrograde ejaculation; occurs when, at orgasm, the ejaculate is forced back into the bladder rather than through the urethra and out the end of the penis
- Erectile dysfunction: the inability to attain and/or maintain an erection suitable for intercourse
- Peyronie's disease (penis problem caused by scar tissue, called plaque, which forms inside the penis. It can result in a bent, rather than straight, erect penis. Most men with Peyronie's disease can still have sex. But for some, it can be painful and cause erectile dysfunction.
- Low sexual desire or loss of libido: a decrease in desire for, or interest in sexual activity, the person's attitude regarding sex is that he or she can "take it or leave it", he/she has neither positive nor negative feelings about sexual expression.[4] Sexual aversion disorders are more frequent in women than in men, this is perhaps due to the higher vulnerability of women to the panic or separation distress.[3]
- Anejaculation (the pathological inability to ejaculate in males, with or without orgasm).

- Painful sex, also known as 'dyspareunia', is pain during or after sex (usually after penetrative sex) Women may also experience painful sex as a result of 'vaginismus', which is when the muscles of the vagina tighten and go into spasm, making penetrative intercourse difficult. This is common in women who:
  - have not had sex before
  - do not know much about sex or are nervous
  - have had a traumatic sexual experience
  - are having relationship problems
  - have another form of vulval or vaginal pain (which can often go undiagnosed)
  - have a condition known as 'endometriosis'
  - have had painful sex in the past

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Symptoms of sexual dysfunction

In men:

- Inability to achieve or maintain an erection suitable for intercourse (erectile dysfunction)
- Absent or delayed ejaculation despite adequate sexual stimulation (retarded ejaculation)
- Inability to control the timing of ejaculation (early or premature ejaculation)

In women:

- Inability to achieve orgasm
- Inadequate vaginal lubrication before and during intercourse
- Inability to relax the vaginal muscles enough to allow intercourse

In men and women:

- Lack of interest in or desire for sex
- Inability to become aroused
- Pain with intercourse

Treatment of sexual dysfunction in males

Sexuality is an important, but often unspoken component of overall life satisfaction; holistic medical care is therefore required.[3]

A thorough sexual history and assessment of general health and other sexual problems (if any) are very important. Assessing performance anxiety, guilt, stress and worry are integral to the optimal management of sexual dysfunction. Many of the sexual dysfunctions that are defined are based on the human sexual response cycle, proposed by William H. Masters and Virginia E. Johnson, and then modified by Helen Singer Kaplan.[5,6]

Due to the interrelatedness of the phases of the sexual response cycle, people with one sexual problem will often manifest other sexual problems. When multiple sexual problems are encountered, try and understand or differentiate the primary problem from the secondary problem.[2]

Treatment of physical causes

- Treating the underlying medical condition
- Change of medication
- Use of prescribed medication
- Life style modifications

Treatment of any psychological causes

- Adequate sexual counseling may improve sexual function of couple
- Sexual education will improve sexual knowledge and participation
- Education on communication would improve partner relationship

Surgical treatment

- Penile implants: inflatable and non-inflatable
- Vascular reconstructive surgery

While sexual problems cannot be prevented, dealing with the underlying causes of the dysfunction can help you better understand and cope with the problem when it occurs. There are some things you can do to help maintain good sexual function:

- Follow your doctor's treatment plan for any medical/ health conditions
- Limit your alcohol intake
- Quit smoking
- Deal with any emotional or psychological issues such as stress, depression, and anxiety. Get treatment as needed.
- Increase communication with your partner[7]

Prognosis

Often, the longer a sexual dysfunction goes on, the more the level of stress and anxiety rises. This can perpetuate the problem. Most of the time, outlook for people with sexual dysfunction is quite good.

However, some medical conditions make it more difficult to overcome.

Conditions caused by stress or temporary circumstances if approached openly can be reversed.

Deep-seated psychological issues may take longer to manage or may never fully resolve, but can be improved.

Sexual dysfunctions & infertility

The relationship between sexual dysfunctions and infertility can be mutual! In other words, sexual dysfunction may cause difficulty conceiving and vice-versa. Disappointment associated with infertility can reduce sexual interest and desire in partners.[4]

Sexual dysfunctions may have pre-existed, even before the infertility problem emerged. In fact, there are couples seeking treatments to resolve the problem of infertility and not their sexual problem, even though they are not sexually active any longer. However, frequent sexual contact is imperative for conceiving, particularly on fertile days. If the couple has rare sexual intercourses due to some dysfunction, then it is very likely that fertile days are missed and, thus, conception is not achieved.

On the other hand, the diagnosis of infertility and assisted reproduction methods often induce high stress and anxiety and are quite 'invasive', both organically and psychologically. Diagnosis and treatment of infertility are usually accompanied with symptoms of dysthymia, depression and tension in the couple's relationship. Repeated failed attempts for conceiving are an additional aggravating psychological factor for the couple.

Time is surely not an ally; on the contrary, it is the biggest enemy. As time passes, the couple's psychological pressure leads to reduction of sexual satisfaction and erotic moments. As a consequence, some sexual dysfunction is very likely to emerge. It is important that individuals/couples who experience this problem see a doctor, psychologist or sex therapist/counsellor to help alleviate some causes.

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Internship for

Trainee Embryologists

Andrology

- Routine semen analysis
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- Semen cryopreservation techniques

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- IVF/ICSI/Micromanipulation techniques
- Daily quality checks in the IVF Laboratory

Learning process

The learning process uses a mix of various methodologies. It includes interactive lectures, seminars, demonstration and laboratory rotations. Interns will be expected to keep a logbook for all procedures observed and those done under minimal or no supervision during the training.

Following the internship, participants who have successfully completed the curriculum will be certified competent in the following;

- Daily laboratory quality checks
- Semen analysis, preparation and freezing
- Oocyte stripping
- Embryo grading and loading for transfer
- Dish preparation for embryo culture and micromanipulation
- Setting up of needles for ICSI/IMSI
- Intracytoplasmic sperm injection

In addition to practical and academic training, each participant will receive:

- A training plan and laboratory protocols
- Logbooks for documentation of all procedures
- World Health Organisation (WHO) Laboratory Manual for the Examination and Processing of Human Semen. 5th Edition, 2010 (electronic copy)
- Daily meal (lunch)
- **Certificate of competency upon completion**

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Educational Qualifications	BSc. Biological Sciences (minimum of 2nd class upper)
Course Duration	6 Months
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- Referees must be able to demonstrate your ability to think critically and logically, application of skills and knowledge in science and social skills. Letter must be properly signed off on a proper letter headed paper.
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For further enquiries, please contact Rose Ogbeche, Head of Laboratory Services Bridge Clinic

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"Risk Management can be summarized as asking and answering three basic questions: What can go wrong? What will we do? And if something happens, how will it be resolved?"

Identifying risks in IVF practice

By Adefemi Olaifa, Clinical Embryologist - Bridge Clinic Fertility Centre, Lagos

Introduction

A risk is any uncertainty about a future event that might threaten an organization's ability to accomplish its mission. It is the chance of something happening that will have a negative impact on an organization's objectives. In particular, risk is the possibility of suffering "loss": loss of quality of outcome, loss of professional regard or profile, loss of referrals, loss of patient/staff health (or even loss of life), loss of profitability, loss of success.

Risk management (RM) is the proactivity in identifying risk and taking appropriate preventive measures to reduce the risk and eliminate the risk. Risk Management was originally an engineering discipline dealing with the possibility that some future events might cause harm. RM includes strategies and techniques for recognizing and confronting any threat and provides a disciplined environment for proactive decision making for the purpose of: Assessing on a continuous basis what can go wrong; determining which risks need to be dealt with; and implementing strategies to deal with these risks.

Risk Management can be summarized as asking and answering three basic questions: What can go wrong? What will we do? (Both to prevent the harm from occurring and in the aftermath of an incident) and If something happens, how will we resolve it?

Consequences of not ensuring proper Risk Management include:

- **More resources will be expended to correct problems that could have been avoided. Catastrophic problems will occur without warning.**
- **There will be no ability to respond rapidly to surprises and recovery will be difficult and costly or even impossible.**
- **The overall possibility of success will be reduced.**
- **The organization will always be in a state of crisis.**[6]

Risk management in the IVF laboratory

Everyday events of any nature always have some element of associated risk; the complex and sophisticated systems involved in successful Assisted Reproductive Technology (ART) inevitably involve a huge catalogue of potential risk factors, and a cascade into catastrophe which can only be prevented or circumvented by acknowledging the risks, cataloguing the elements surrounding them, establishing a system of active risk management that recognizes and acknowledges influence and responsibility for events in the future.

ART is a field of human endeavour that uniquely and completely depends upon multidisciplinary cooperation and collaboration. IVF risk factors include clinical, laboratory, legal/regulatory, administration and ethical situations.

A comprehensive Quality Management Systems (QMS) is a first line of defence against potential risks in the IVF laboratory encompassing training, protocols and procedures, good system of communication and feedback, witnessing and back-up systems.

Once risks are identified, the laboratory must implement continuous monitoring and control processes to minimize or reduce the risks.[2]

In this article we shall be focusing on risk management in the IVF laboratory.

IVF Laboratory risk factors and management

Laboratory Staff: An optimal IVF laboratory requires careful management to ensure that workload is safe, staff training with regular review is effectively in place, and work is allocated according to established and recognized competencies.

Safe workload for embryologist ranges between 100 to 150 cycles per year per embryologist depending upon the range and complexity of treatments offered, and the levels of training and experienced available staff.

A training logbook is usually kept by the trainee to provide a record of the tasks completed, with an area to note any mistakes, together with corrective action taken.

After sufficient training, the trainee should demonstrate that he/she can competently perform a task, under full supervision by a senior member of staff.

Competence can also be evaluated with the help of Quality assessment schedules such as UKNEQAS (www.ukneqas.org.uk) and fertAid (www.fertaid.com). This has additional benefit of allowing comparisons with other centres.[2]

Equipment: The deterioration of equipment is normal, and this process begins as soon as the equipment is installed. If deterioration is not checked, it can cause electrical failures and malfunctions. Therefore, equipment used in the IVF laboratory should be maintained periodically and preferably every six months. Maintenance log and report of each equipment should be kept. Properly maintained equipment reduces downtime by minimizing catastrophic failures.[5]

Managing the equipment risk factor in the IVF laboratory also involves ensuring that at least a spare of all equipment is available in case of equipment breakdown.

IVF laboratory procedures should not start each day without doing daily quality index (DQI).

Daily Quality Index is the daily check of all equipment used in the IVF laboratory before the start of procedure each day to ensure that the equipment are functioning optimally. Checks of functional parameters for devices used to maintain temperature and CO2 should be performed using calibrated thermometers and extra methods of CO2 analysis and/or pH measurement. Record of these measurements, as well as those shown by the digital displays of each device, must be retained. (Magli et al., 2008)

Power: An onsite generator should be available for back-up power to the laboratory, and as well with all critical equipment maintained regularly. All equipment in the IVF laboratory should be connected to uninterrupted power supply (UPS) so that in the event of power failure or fluctuations can back up power supply.[2]

Culture Media: Media management and supply is a very critical risk factor in the IVF laboratory due to its sensitivity and importance to the success of the IVF laboratory. Culture media may not be readily available when needed, so a lot of planning must go into its management to prevent culture media stock out. Reorder levels must be strictly adhered to. Culture media should be of tissue culture grade, preferably mouse embryo tested and with purity appropriate for the purpose. Use of commercially produced, quality controlled tested media is recommended. When commercially produced media are used, it is important to check that producers use validated quality control testing, if not this has to be done by the laboratory.[2] In addition, integrity of the packages and appropriate delivery conditions should be controlled. All culture media shipment received must be accompanied with data loggers that track the temperature of the media during transit.

The pH of all culture media received should be assessed using a pH measuring device and document the reading. The normal range for pH is between 7.2 and 7.5.

Consumables: All consumables used in the IVF laboratory should be CE marked and this signifies that the products have been assessed to meet high safety, health, and environmental protection requirements and the consumable should also be mouse embryo assay (MEA) tested. MEA testing screens the media and materials for toxicity prior to lot release.

Patients: Systematic screening for syphilis, HIV, HBV and HCV for all IVF clients is very important. This infection screening helps to manage the risk of infection in the IVF laboratory and all staff involved will take note and take appropriate precautions when working with the samples.[4]

The risk that cross-contamination with infectious material from one patient to another could occur in storage. It is advisable to keep the material stored in the cryopreservation tanks in a way that avoids contact of the liquid nitrogen phase with the biological substances. To further minimise the risk of cross-infections, the infected samples should be stored in a dedicated tank for samples of patients with the same infection.

Testing of all patients prior to treatment as a requirement from the European Directive 2004/23/EC and Commission Directives will drastically reduce the potential risk of cross-contamination during all procedures, including cryopreservation (Magli et al., 2008).

Organization: Active risk management can identify failures in organization/systems by keeping meticulous records that can be objectively examined on a regular or routine basis.[2]

The most high profile risk is that of confusion or mix-ups in the IVF laboratory between gametes or embryos of different couples. Adherence to principles of good laboratory practice in IVF laboratory should ensure that there is only a minimal chance of mix-ups between gametes and embryos of different couples. First of all, all containers used for processing gametes and embryos must be clearly and indelibly labelled with as much identifying information as possible. In 2002, Human Fertilization and Embryology Authority (HFEA) introduced mandatory witnessing for all IVF laboratory procedures and allowed electronic witnessing in 2006. Therefore, double checking should be performed on all clinical and laboratory procedures.[1]

Standard Operating Procedure (SOP) should be available for all procedures and easily accessible as well. The SOPs must be clearly written and easy to understand outlining every step to be taken in carrying out each procedure. Introducing new techniques or changing existing protocols always involves risks: these can be minimized by making sure that it is not a unilateral decision, and that everyone is aware of and has agreed to the procedure.[2]

To minimize the risk of error during identification of patients and cell manipulation, an effective and accurate traceability system able to uniquely locate gametes and embryos during each step of the IVF procedure, from procurement to disposition, and vice versa, is mandatory. A proper identification system should also ensure that information on the main characteristics of patients, cycle and cells, together with relevant data related to products and materials coming into contact should be provided continuously.[1]

Disaster Management: Some parts of the world are more prone to perils or natural disaster than others: "Acts of God," which are beyond the scope of human preventive action. Severe weather, earth quakes, tsunamis, volcanoes, forest fires, and civil disturbances can all pose a threat to the safety of gametes and embryos in the laboratory, as well as staff and patients. Detailed Contingency plans to protect the gamete/embryos in culture and frozen embryos should be put in place.

Conclusion

Risk management helps to change the systems and processes which brings improvement to the system. Risk management can also minimize the possibility of errors occurrence and ensures accuracy in ART outcome.

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"Paired-end MPSS allows accurate digital counting while also determining the length of each cfDNA fragment by sequencing both its extremities[8]"

Performance of the neoBona test: a new paired-end massively parallel shotgun sequencing approach for cell-free DNA-based aneuploidy screening

Ultrasound Obstet Gynecol 2017 Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/uog.17386.

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Keywords

cell-free DNA | trisomy | screening | paired-end sequencing

Abstract

Objective To assess the performance of screening for fetal trisomies 21, 18 and 13 by cell-free (cf) DNA analysis of maternal blood using a new method based on paired-end massively parallel shotgun sequencing (MPSS).

Methods This was a blinded study of plasma samples (1mL) obtained from 1000 women undergoing screening for fetal trisomies 21, 18 and 13 at 11–13 weeks' gestation. The study included 50 cases with confirmed fetal trisomy 21, 30 with trisomy 18, 10 with trisomy 13 and 910 unaffected pregnancies. Paired-end MPSS with the neoBona test allowed simultaneous assessment of fetal fraction, cfDNA fragment size distribution and chromosome counting, which were integrated into a new analysis algorithm to calculate trisomy likelihood ratios (t-score) for each chromosome of interest. Each sample was classified as trisomic or unaffected using chromosome-specific cut-offs set at t-score values of 1.5 for trisomy 21 and 3.0 for trisomies 18 and 13.

Results Valid results were provided for 988 (98.8%) cases; 12 (1.2%) samples, from nine euploid and three trisomy 21 pregnancies, did not pass quality-control criteria and were excluded from further analysis. All 47 cases of trisomy 21, all 10 of trisomy 13, 29 of 30 with trisomy 18 and all 901 unaffected cases were classified correctly. Median fetal fraction was 10.5% (range, 0.3–33.8%) and trisomic and unaffected cases with low fetal fractions of<1% were identified correctly.

Conclusions This novel method for cfDNA analysis of maternal plasma, which utilizes paired-end MPSS, can provide accurate prediction of fetal trisomies. Use of a new multicomponent t-score removes the need to reject samples with fetal fraction <4%, which potentially extends the benefits of non-invasive prenatal cfDNA analysis to a larger proportion of pregnancies.

Introduction

Screening for fetal aneuploidy by analysis of cell-free (cf) DNA in maternal blood was made possible by the advent of massively parallel shotgun sequencing (MPSS) which allows digital counting of cfDNA fragments, either by whole-genome sequencing[1,2] or targeted approaches[3,4]. Detection of fetal trisomy using counting statistics, such as Z-score or normalized chromosome values (NCV), becomes easier and more robust when the proportion of fetal DNA to total cfDNA in maternal blood is high because of greater separation between normal and aneuploid cases[5,6]. The sensitivity of detecting fetal trisomies at low fetal fraction is dependent mostly on the amount of useful counts of the chromosome of interest or sequencing depth[6,7]. Inclusion of fetal fraction in analysis algorithms can improve specificity because, in cases of low Z-scores or NCVs, it helps distinguish between aneuploid cases with low fetal fraction from euploid cases with a higher fetal fraction[5].

Paired-end MPSS allows accurate digital counting while also determining the length of each cfDNA fragment by sequencing both its extremities[8]. As cfDNA fragments of fetal origin are slightly shorter than maternal ones, size differences can be used to determine fetal fraction[8]. Additionally, in the case of fetal aneuploidy, counting differences detected from all cfDNA fragments would appear more evident if confirmed on shorter fragments only[8,9].

neoBona (Labco Diagnostics, Barcelona, Spain) is the first cfDNA-based screening test to exploit paired-end MPSS through a novel bioinformatics approach, which has the advantage of combining conventional counting statistics with the distribution of cfDNA fragment size to provide a double check of chromosome counting data. Additionally, by integrating sequencing depth on each chromosome and fetal fraction it allows calculation of a unique trisomy score (t-score), thereby quantifying the likelihood of fetal trisomy. The objective of this study was to evaluate the performance of this new method on a large blinded set of archived maternal plasma samples, tested without previous knowledge of their outcomes.

Methods

Study population

Blood samples were collected between April 2006 and February 2015 at King's College Hospital, London, UK, from women with a singleton pregnancy undergoing screening for trisomies 21, 18 and 13 by assessment of a combination of fetal nuchal translucency (NT) thickness and maternal serum free beta human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11–13weeks' gestation[10]. Gestational age was determined from measurement of the fetal crown–rump length[11]. Women with a high risk from the combined test had chorionic villus sampling (CVS) for fetal karyotyping. Karyotype results obtained from genetic laboratories and details on pregnancy outcome obtained from the maternity computerized records or the general medical practitioners of the women were added into the database as soon as they became available.

All patients gave written informed consent to provide samples for research, which was approved by the National Health Service Research Ethics Committee.

Blood samples were collected into EDTA BD vacutainer™ tubes (Becton Dickinson UK limited, Oxford, UK) and centrifuged at 2000g for 10min within 15min of collection (Plasma 1) followed by another 10min at 16000g to further separate cell debris (Plasma 2). Samples of Plasma 1 and 2 were divided into 0.5-mL aliquots in separate Eppendorf tubes, labelled with a unique patient identifier and stored at −80°C for up to 9 years until analysis. A total of 50 cases with trisomy 21, 30 with trisomy 18, 10 with trisomy 13 and 910 normal controls with 1mL of Plasma 1 or 2 were selected for analysis. Cases with aneuploidy were selected at random and each was matched to 11–12 controls that were sampled on the same or next day as the aneuploid case. Normal controls were uncomplicated pregnancies resulting in live birth after 38 weeks' gestation of pheno-typically normal neonates assumed to be euploid. None of the samples was previously thawed and refrozen.

Plasma samples (two tubes of 0.5mL per patient) were coded and sent on dry ice from London to the central laboratory of Labco Diagnostics in Barcelona, Spain, where blinded cfDNA analysis was performed using the neoBona test.

Analysis of samples

The only information provided to the laboratory for each sample was the patient-unique identifier ,date of collection and whether it was a Plasma 1 or 2 sample. Each sample was assessed for volume, adequacy of labeling and risk of contamination or sample mixing before evaluation of fetal trisomy. Although the volume was<1mL (range, 500–950µL) in 60 of the 1000 samples, they were included in the analysis. Plasma samples from each patient were collected into 96 deep-well plates. Plates of Plasma 1 samples underwent a second centrifugation step at 16000g before DNA extraction. Samples were processed in batches of 96 using VeriSeq NIPT v1.0 chemistry (Illumina Inc, San Diego, CA, USA) on a fully automated workstation (Hamilton Star, Hamilton, Reno, NV, USA) designed to handle plasma isolation, column-based DNA extraction, set-up of sequencing library, quantification, normalization and pooling. Sequencing libraries from each batch of 96 samples were collected in two separate pools of 48 double-indexed samples which underwent paired-end MPSS for two sets of 36 cycles using NextSeq 500 and 550 sequencers with TG NextSeq 500/550 High Output Kit v1.2 (Illumina inc). Sequencing outputs were analyzed using the VeriSeq NIPT software v1.0 (Illumina inc).

After de-multiplexing and filtering, sequence alignment was performed against HG19 for data normalization and interchromosome comparisons[7]. Regions affected by poor alignment were filtered out and further normalization was applied based on a principal component decomposition as described by Zhao et al.[12]. Fetal fraction assessment, based on molecular size distributions and differences in coverage between fetal and maternal cfDNA, was complemented with X and Y chromosomes data in cases of male fetuses[8,9,13]. NCVs were calculated for chromosomes 13, 18 and 21, as described previously [6,14]. NCV counting statistics are similar in principle to the conventional Z-score, with a fixed cut-off of around 3.0 to discriminate between trisomic and unaffected pregnancies, the main difference being that, for NCVs, each chromosome of interest is only normalized against a specific set of chromosomes, optimized for comparable sequencing coverage to minimize variations.

Trisomy likelihood ratios (t-scores) for each chromosome of interest were calculated for each sample based on the estimated fetal fraction, counting statistics (NCVs) derived from both total and short DNA fragments, and sequencing depth. The likelihood ratio reflects the probability for a sample to be affected, given the observed counting statistics and fetal fraction, versus the probability of a sample to be unaffected, given the same counting data. Thus, using this analysis approach, trisomic samples with low fetal fraction can result in a higher t-score if they have, for instance, a higher depth of sequencing enabling efficient counting on short DNA fragments which are mostly of fetal origin.

Samples were classified as being compatible with the presence or absence of trisomy 21, 18 or 13 using predefined chromosome specific cut-offs at t-score values of 1.5 for trisomy 21 and 3.0 for trisomies 18 and 13.

Quality-control analyses (QCs) were applied to monitor sequencing depth, the distribution of cfDNA fragment sizes, sequencing coverage for chromosome denominators and for the estimate of fetal fraction. Results were considered valid only for samples passing all QCs.

Results were provided to King's College Hospital in which the classification for each case was compared to pregnancy outcome and detection rates and false-positive rates were estimated.

Results

The characteristics of the study population are summarized in Table 1. Compared to euploid pregnancies, in pregnancies with trisomy 21, median maternal age, fetal NT and serum free β-hCG were higher and serum PAPP-A was lower and in pregnancies with trisomy 18 or 13 median maternal age and fetal NT were higher and serum free β-hCG and PAPP-A were lower.

The cfDNA test provided results for 988 (98.8%) cases. In total, 12 (1.2%) samples, nine from euploid and three from trisomy 21 pregnancies, failed to provide a result and were excluded from further analysis. The reasons for QC failure were size distribution of cfDNA fragments beyond the expected range (n=6), low sequencing depth for the observed fetal fraction (n=4), unusually high DNA concentration (n=1) and insufficient sequencing coverage for determination of fetal fraction (n=1).

Table 1 Characteristics of 1000 pregnant women undergoing prenatal screening for fetal trisomies, according to outcome

Characteristic	Euploid (n=910)	Trisomy 21 (n=50)	Trisomy 18 (n=30)	Trisomy 13 (n=10)
Maternal age (years)	31.9 (27.3–34.9)	37.9 (35.3–41.3)	35.4 (28.8–40.5)	33.5 (30.0–34.9)
Maternal weight (kg)	65.0 (59.0–75.0)	68.0 (60.7–75.0)	66.8 (60.4–75.5)	64.5 (60.6–64.9)
Maternal height (cm)	165 (160–169)	166 (161–172)	166 (160–171)	167 (164–170)
Racial origin				
Caucasian	563 (61.9)	41 (82.0)	17 (56.7)	8 (80.0)
Afro-Caribbean	244 (26.8)	6 (12.0)	6 (20.0)	1 (10.0)
South Asian	32 (3.5)	1 (2.0)	3 (10.0)	0 (0)
East Asian	26 (2.9)	2 (4.0)	2 (6.7)	0 (0)
Mixed	45 (4.9)	0 (0)	2 (6.7)	1 (10.0)
Cigarette smoker	55 (6.0)	3 (6.0)	1 (3.3)	1 (10.0)
Method of conception				
Spontaneous	880 (96.7)	46 (92.0)	26 (86.7)	10 (100)
Ovulation drugs	9 (1.0)	3 (6.0)	2 (6.7)	0 (0)
In-vitro fertilization	21 (2.3)	1 (2.0)	2 (6.7)	0 (0)
Fetal crown–rump length (mm)	61.8 (57.0–67.6)	66.1 (60.0–73.0)	56.1 (51.9–61.6)	59.0 (51.1–63.1)
GA at screening (weeks)	12.6 (12.2–13.0)	12.9 (12.5–13.4)	12.2 (11.8–12.6)	12.4 (11.8–12.7)
Fetal NT thickness (mm)	1.7 (1.5–1.9)	4.4 (3.4–6.2)	6.5 (3.6–7.9)	5.2 (2.3–6.3)
PAPP-A MoM	1.126 (0.766–1.563)	0.695 (0.441–0.869)	0.227 (0.135–0.327)	0.371 (0.282–0.570)
Free β-hCG MoM	0.995 (0.678–1.582)	2.259 (1.574–3.109)	0.293 (0.171–0.362)	0.314 (0.204–0.747)

Data are given as median (interquartile range) or n (%). β-hCG, beta human chorionic gonadotropin; GA, gestational age; MoM, multiples of the median; NT, nuchal translucency; PAPP-A, pregnancy-associated plasma protein-A.

Table 2 Results of cell-free DNA analysis by neoBona test for fetal trisomy screening in 988 women with test result, according to outcome

Result	Euploid (n=910)	Trisomy 21 (n=47)	Trisomy 18 (n=30)	Trisomy 13 (n=10)
NCV for chromosome 21	−0.01 (−3.43 to 3.55)	11.50 (3.59 to 25.67)	0.32 (−1.88 to 3.48)	−0.048 (−1.36 to 1.25)
t-score for trisomy 21	−23.2 (−1074.2 to 0.6)	101.0 (7.2 to 392.1)	−12.0 (−178.4 to −1.1)	−20.5 (−121.8 to −5.2)
NCV for chromosome 18	0.01 (−3.25 to 6.17)	−0.24 (−2.42 to 2.71)	12.24 (−1.22*to 36.91)	0.72 (−1.49 to 2.56)
t-score for trisomy 18	−31.3 (−1960.6 to 1.0)	−38.6 (−322.2 to −3.6)	94.5 (−17.9*to 765.2)	−21.9 (−247.2 to −7.2)
NCV for chromosome 13	0.001 (−4.55 to 4.44)	−0.09 (−1.91 to 2.58)	0.42 (−2.30 to 2.16)	14.76 (6.31 to 28.50)
t-score for trisomy 13	−37.6 (−2591.6 to 0.1)	−48.6 (−449.6 to −1.3)	−15.2 (−442.6 to 2.0)	209.3 (23.9 to 479.5)
Fetal fraction (%)	10.2 (0.3 to 33.8)	10.7 (3.8 to 19.8)	9.6 (0.8 to 23.0)	7.9 (4.0 to 15.3)
Trisomy 21t-score>1.5	0 (0)	47 (100)	0 (0)	0 (0)
Trisomy 18t-score>3.0	0 (0)	0 (0)	29 (96.7)	0 (0)
Trisomy 13t-score>3.0	0 (0)	0 (0)	0 (0)	10 (100)

Data are given as median (range) or n (%). *Value from the only discrepant result; case of trisomy 18 with 11% fetal fraction resulted in trisomy likelihood score (t-score) and normalized chromosome value (NCV) compatible with normal chromosome 18 copy number.

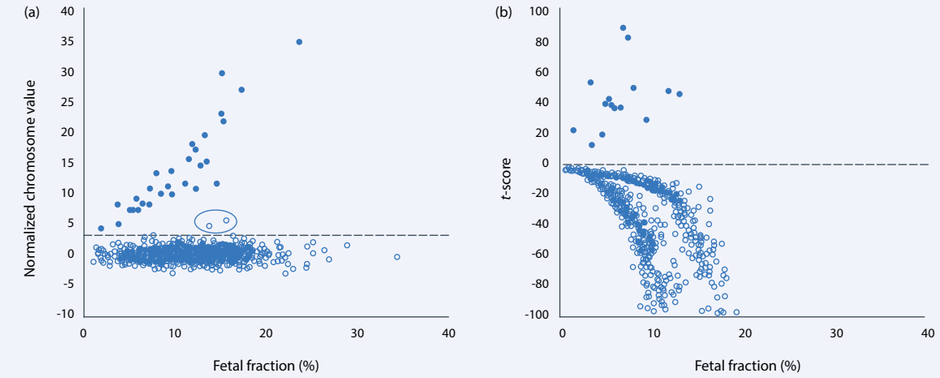

Figure 1 Normalized chromosome value (NCV) (a) and trisomy likelihood score (t-score), for values between −100 and 100 (b), for chromosome 18, in 29 pregnancies with trisomy 18 (●) and 901 unaffected pregnancies (○), plotted against fetal fraction. Plot of NCV in (a) shows limitation of this method because, in two cases of trisomy 18 with low fetal fraction (<4%) (arrows in (a) and (b)), NCV was similar to those of two unaffected cases (circled) with high fetal fraction; these unaffected cases would have been classified wrongly as positive for trisomy 18 as they are above the cut-off of 3.0 (----). Plot of t-scores in (b) shows cases of trisomy 18 with score well above cut-off of 3.0 for trisomy 18 (----), including one case with fetal fraction <1%.

The cfDNA test classified correctly all 47 pregnancies with fetal trisomy 21, all 10 with trisomy 13, 29 (96.7%) of 30 with trisomy 18 and all 901 unaffected pregnancies (Table 2). In one case of trisomy 18, t-score and NCV values for chromosome 18 were compatible with normal chromosome copy number; in this case the fetal fraction was 11%. One case with trisomy 21 and one unaffected pregnancy had the same NCV of 3.5, but had different t-scores for trisomy 21 which were 10 and −14, respectively. Therefore, using the predefined cut-offs of t-score values of 1.5 for trisomy 21 and 3.0 for both trisomies 18 and 13 resulted in detection rates of 100% for trisomies 21 and 13 and 96.7% for trisomy 18, with false-positive rate of 0% for all trisomies.

The mean fetal fraction was 10.6% for euploid pregnancies, 11.1% for trisomy 21, 9.4% for trisomy 18 and 8.9% for trisomy 13. One case of trisomy 21, three of trisomy 18 and 58 unaffected pregnancies were identified correctly despite showing fetal fractions below 4%, including one case of trisomy 18 and nine euploid cases with fetal fraction <1% (Table 2 and Figure 1).

Discussion

The findings of this study demonstrate the feasibility of a new approach for cfDNA testing of maternal blood in screening for fetal trisomies 21, 18 and 13. Paired-end MPSS of cfDNA coupled with a novel analysis algorithm provided simultaneous assessment of fetal fraction, distribution of size of DNA fragments and chromosome counting. Trisomy likelihood ratios for each chromosome of interest could then be calculated for each sample based on the estimated fetal fraction, chromosome-specific counting statistics on total and short fragments and sequencing depth. We used this novel approach to examine stored plasma samples and, at preselected chromosome-specific cut-offs of t-score values of 1.5 for trisomy 21 and 3.0 for trisomies 18 and 13, the test classified correctly all cases of trisomy 21, trisomy 13 and unaffected pregnancies and 29 of 30 cases of trisomy 18. Such high performance of screening is compatible with the best results of previous studies utilizing cfDNA testing to screen for trisomies 21, 18 and 13[15].

In the single case of trisomy 18 that was misclassified, the fetal fraction was 11% and is therefore highly unlikely that this error was related to technical issues affecting test sensitivity. Unfortunately, no more sample was available to repeat the test and exclude errors due to laboratory mishandling. In addition, trisomy rescue, generating a normal cell line in the cytotrophoblast, could not be ruled out as the underlying cause of this discrepancy as prenatal diagnosis was only performed on long-term CVS culture by quantitative fluorescent polymerase chain reaction and karyotype, but not on direct preparation.

The basis for cfDNA testing using counting methods is that, in trisomic pregnancies, the number of molecules derived from the extra fetal chromosome, as a proportion of all sequenced molecules in maternal plasma, is higher than in euploid pregnancies. The ability to detect the small increase in the amount of a given chromosome in maternal plasma in a trisomic compared to a disomic pregnancy is related directly to the fetal fraction and the depth of sequencing[3,16–19]. Trisomy cases with low fetal fraction used to be more difficult to discriminate from normal samples by counting statistics only, as they can produce NCVs with similar values to those occasionally observed in normal samples with higher fetal fraction[6], thus reducing test specificity. Also the sensitivity could be affected if, for the sequencing depth used, the proportion of fetal cfDNA is too low to allow discrimination of trisomies by counting statistics only[7,18]. For these reasons, when the fetal fraction is below 4%, which occurs in 0.5–6.1% of pregnancies, the cfDNA test is usually presented as a failure and no result is reported[15].

Some of the problems due to low fetal fraction have now been overcome by the application of the multicomponent t-score, as the resolution in discriminating between tri-somic and unaffected pregnancies is no longer dependent only on fetal fraction but also on the new possibility of performing additional counting statistics on short DNA fragments,which are mostly of fetal origin. Consequently, trisomic pregnancies with low fetal fraction could result in higher t-score values than in pregnancies with higher fetal fraction and lower total sequencing depth or less efficient counting statistics on short fragments. This approach proved to be highly efficient at low fetal cfDNA amounts, as all four aneuploid cases with fetal fraction between 0.8% and 3.5% could be detected. The effectiveness of the new multicomponent t-score to improve overall specificity was evident in one case of trisomy 21 and one unaffected sample that were classified correctly despite generating the same NCV, and thus would be undistinguishable by conventional MPSS analysis algorithms.

Despite testing archived plasma samples, and with suboptimal volumes in 6% of cases, failure to provide a result was only observed in 1.2% of samples. The most common reason for test failure was an abnormal distribution of size of cfDNA fragments, which affected size-based counting and the measurement of fetal fraction. This artifact was likely to be caused by cfDNA shearing, resulting from sample degradation. It is therefore expected to occur less frequently in routine clinical samples collected in dedicated tubes, designed to prevent cell lysis and stabilize cfDNA. Four more samples failed the QC analysis which combines sequencing metrics and estimated fetal fraction, thus determining if the analysis output has statistical confidence in scoring a sample. Repeating the test on a second aliquot of the same plasma would probably have yielded a valid result. In clinical routine, the test is usually performed within a few days from sampling and with enough volume to be repeated, therefore this technical failure is expected to decrease.

The novel approach presented in this study has the potential of extending the advantages of cfDNA-based aneuploidy screening to a wider proportion of pregnancies. Complementing conventional counting statistics with size-based chromosome counting and fetal fraction ensured that accurate prediction of trisomic status was provided in 62 of our cases with fetal fraction <4%. Consequently, it is no longer necessary to exclude samples from analysis solely because the fetal fraction is <4% if enough sequencing depth is reached for the corresponding amount of cfDNA and size-based counting is performed at the same time.

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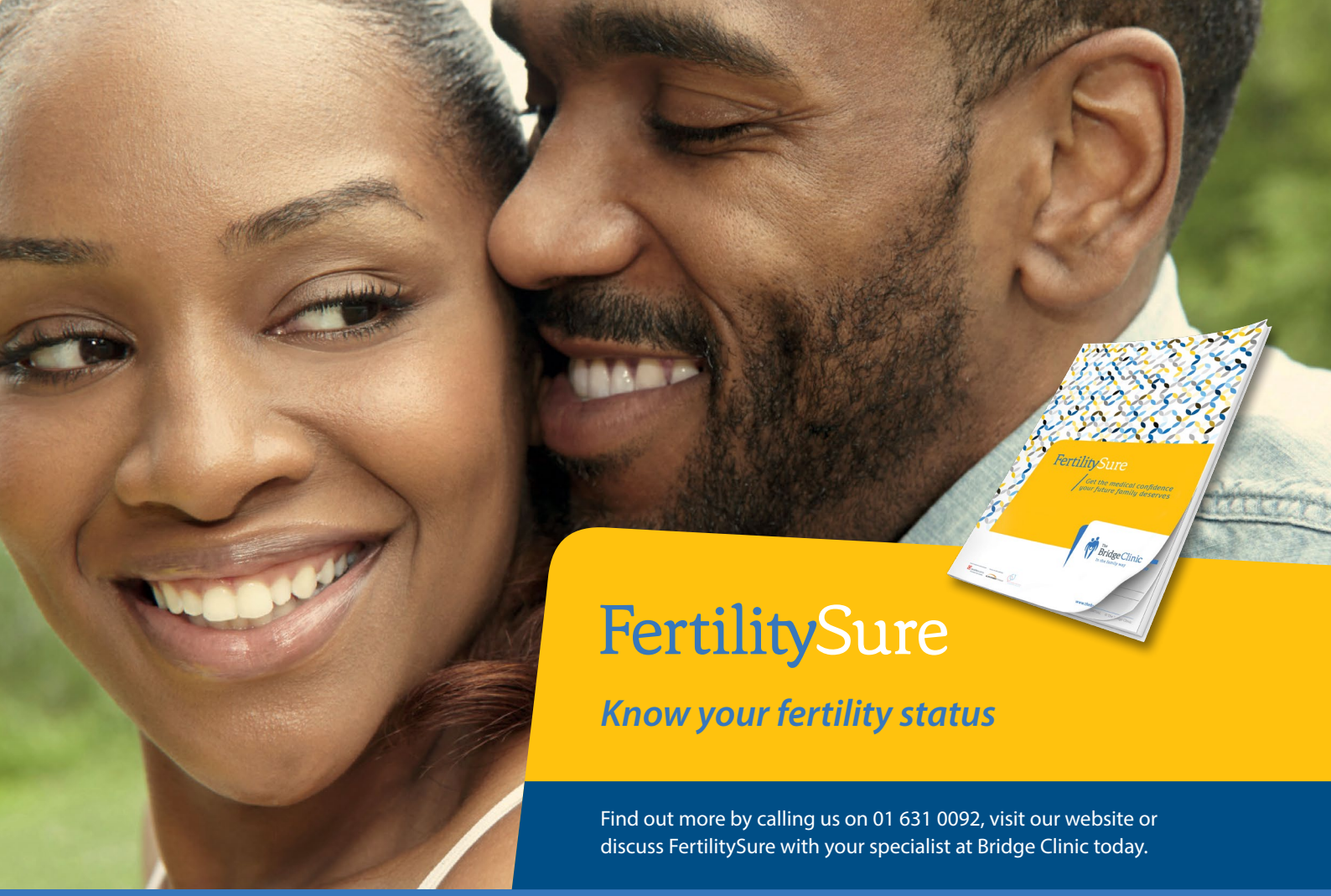

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