



UNIVERSITY OF  
BIRMINGHAM

**IMSR**   
INSTITUTE OF METABOLISM  
AND SYSTEMS RESEARCH

**Tommy's**

National Centre for  
Miscarriage Research

**NHS**

Birmingham Women's and Children's  
NHS Foundation Trust

# **The role of hydrosalpinx in recurrent miscarriage**

## **PROTOCOL**

### **Version 1.1**

**28th December 2017**

#### **Chief Investigator**

Professor Arri Coomarasamy

Tel: 0121 627 2775

Email: [a.coomarasamy@bham.ac.uk](mailto:a.coomarasamy@bham.ac.uk)

#### **Key contacts:**

- The CI is responsible for the medical oversight of the study.
- Professor Arri Coomarasamy is the Director of the Tommy's National Centre for Miscarriage Research
- Dr. Adam Devall is the Trial Manager for Tommy's National Centre for Miscarriage Research: 0797 182 3452; [a.j.devall@bham.ac.uk](mailto:a.j.devall@bham.ac.uk)
- Dr. Sean Jennings is the Research Governance and Ethics Manager for the University of Birmingham (Sponsor): 0121 414 3794; [researchgovernance@contacts.bham.ac.uk](mailto:researchgovernance@contacts.bham.ac.uk)

# Table of Contents

The role of hydrosalpinx in recurrent miscarriage study design group.....	3
Principal Investigator & Chief Investigator .....	3
Study Design and Management.....	3
Co-Investigators .....	3
Background .....	4
<i>Epidemiology of miscarriage</i> .....	4
Tubal disease and its effect on reproductive outcomes.....	4
Detection of tubal disease .....	5
Rationale for this study .....	5
Objectives .....	6
Study Design .....	6
Population to be studied.....	6
Inclusion criteria.....	6
Exclusion criteria .....	6
Identifying Participants .....	6
Data to be collected and tests to be performed.....	6
Outcomes to be measured .....	7
Method of collection of data and outcomes .....	7
Consent to participation and feedback of results.....	8
Dissemination of study results.....	8
Withdrawal process .....	8
Patient Journey .....	8
Accrual and Analysis .....	9
Sample Size .....	9
Statistical Analysis.....	9
Data management and archiving.....	9
Safety reporting (Adverse Events and Serious Adverse Events).....	10
Serious breaches.....	10
Insurance and indemnity .....	10
End of study definition.....	10
Study Oversight and Quality Assurance processes.....	10
Funding and Sponsorship.....	10
Study Flow Chart.....	12
List of References.....	13

# The role of hydrosalpinx in recurrent miscarriage study design group

## Principal Investigator & Chief Investigator

Professor Arri Coomarasamy  
Professor of Gynaecology, University of Birmingham  
Birmingham Women's Hospital  
Telephone: 0121 627 2775  
[a.coomarasamy@bham.ac.uk](mailto:a.coomarasamy@bham.ac.uk)

## Study Design and Management

Dr Jayasish Ghosh  
Clinical Research Fellow and Registrar in Obstetrics and Gynaecology  
Birmingham Women's Hospital  
Telephone: 0121 623 6837  
[jayasish.ghosh@nhs.net](mailto:jayasish.ghosh@nhs.net)

## Co-Investigators

Dr Ioannis D Gallos  
Honorary Clinical Lecturer and Sub-speciality Trainee in Reproductive Medicine and Surgery  
Birmingham Women's Hospital  
Telephone: 0121 623 6837  
[I.D.Gallos@bham.ac.uk](mailto:I.D.Gallos@bham.ac.uk)

Dr Justin J Chu  
Academic Clinical Lecturer and Registrar in Obstetrics and Gynaecology  
Birmingham Women's Hospital  
Telephone: 0121 623 6837  
[justinchu@doctors.org.uk](mailto:justinchu@doctors.org.uk)

# Background

## *Epidemiology of miscarriage*

Miscarriage, also known as spontaneous pregnancy loss before the fetus reaches viability, is a common early pregnancy complication. One in five pregnancies miscarries, which equates to over 250,000 miscarriages a year and 38,349 hospital admissions in the UK between April 1<sup>st</sup> 2015 and March 31<sup>st</sup> 2016.<sup>1,7</sup> Miscarriage can be devastating to women and couples trying to conceive. It is a significant source of physical and psychological morbidity for many women and couples, the effects of which can endure for many months.<sup>8</sup> Yet for all the physical and mental sequelae and the economic costs, we have made little progress in treating this distressing condition.

Most miscarriages happen in the first 12 weeks of pregnancy because of sporadic chromosomal abnormalities.<sup>9</sup> However, for women with recurrent miscarriages, there may be genetic factors such as parental chromosomal abnormalities, embryonic chromosomal abnormalities, as well as structural anatomical abnormalities such as congenital uterine malformations causing miscarriage.<sup>10-17</sup> Other risk factors include endocrine disease such as diabetes, systemic and genital infections and thrombophilia such as Anti-Phospholipid Syndrome.<sup>18,19</sup> The full list is recorded in appendix A for reference. However, despite very thorough investigation (full list of recommended investigations is outlined in Appendix B), a significant proportion of women with recurrent miscarriage will be labelled as unexplained and be left with no definitive answers, treatments or sometimes sadly, hope.<sup>20</sup>

## *Tubal disease and its effect on reproductive outcomes*

*C. trachomatis* is the most common sexually transmitted infection in the UK.<sup>2</sup> *C. trachomatis* often has no symptoms, but can have serious health consequences, including fallopian tube damage. Untreated *C. trachomatis* infection in pregnancy may increase the risk of miscarriage, preterm birth, low birth weight and stillbirth.<sup>4-6</sup>

A severe form of tubal damage is when the tube is blocked and filled with fluid, a condition called hydrosalpinx. They are classically caused by ascending infections from the lower genital tract such as chlamydia, which cause pelvic inflammatory disease, causing the tubes to appear swollen and enlarged.<sup>21</sup> Hydrosalpinx is associated with a dramatic reduction in the chances of a live birth with assisted reproductive techniques.<sup>22</sup> This is explained by reduced implantation chances and increased pregnancy losses in the presence of a hydrosalpinx.<sup>22</sup> It is therefore strongly recommended that hydrosalpinges are removed or treated by tubal surgery prior to IVF.<sup>23</sup>

It has been suggested that hydrosalpinx may have a causative role in recurrent miscarriage. The biological rationale for this increase in pregnancy losses is not yet well understood. It has been postulated that the hydrosalpinx fluid may be embryotoxic as it can include micro-organisms, endotoxins, cytokines and free radicals causing oxidative stress.<sup>24,25</sup> This fluid may therefore alter the endometrial receptivity causing a disturbed cytokine cascade, which impedes implantation.<sup>26</sup> Additionally, as the fallopian tube is occluded distally, the hydrosalpinx fluid may drain into the endometrial cavity having a flushing effect on the developing embryo hindering implantation.<sup>27</sup>

## Detection of tubal disease

Investigation of suspected tubal disease is currently recommended for women with subfertility by laparoscopy or hysterosalpingography (HSG).<sup>23</sup> Women who are not known to have comorbidities (such as pelvic inflammatory disease, previous ectopic pregnancy or endometriosis) should be offered HSG to screen for tubal occlusion because this is a reliable test for ruling out tubal occlusion, and it is less invasive and makes more efficient use of resources than laparoscopy.<sup>23</sup> A HSG involves the passage of a radio-opaque contrast media through the female cervix whilst simultaneously taking serial radiographs to examine the patency and free spill of the media into the abdominal cavity. It requires radiographers, specialist radiographic equipment and exposure to radiation. Furthermore, a HSG is performed in a radiology department and requires a radiologist to interpret them.

Where appropriate expertise is available, screening for tubal occlusion using hysterosalpingo-contrast-ultrasonography (HyCoSy) can be considered because it is an effective alternative to HSG for women who are not known to have comorbidities.<sup>23</sup> A HyCoSy is an outpatient procedure which only requires a special contrast media and an USS machine which is usually present in most gynaecological out-patient departments. It is performed at the same time as a pelvic ultrasound, which is recommended for both women with subfertility or recurrent miscarriages.<sup>20,23</sup> The contrast media is injected through the cervix and its flow into the uterus and out through the fallopian tubes is tracked in real time using the ultrasound machine to detect tubal patency and pathology. A HyCoSy is a standard investigation for fertility problems.

Laparoscopy is an invasive operative investigation where by a fibre optic camera inserted through the umbilicus is used to directly visualise the passage of dye through the fallopian tubes thereby confirming their patency and function. Women who are thought to have comorbidities should be offered laparoscopy and dye so that tubal and other pelvic pathology can be assessed and treated at the same time. Laparoscopy costs the NHS significantly more than HSG or HyCoSy and carries more risk. Costs associated with laparoscopy are in the form of theatre use and staffing, equipment used, recovery facilities and a ward bed.

In addition to the tubal investigations mentioned above, it is also necessary to identify whether women undergoing these investigations have concurrent genital tract infection before undertaking tubal assessment. Tests to diagnose genital *C. trachomatis* infections have evolved rapidly and nucleic acid amplification tests (NAAT), such as polymerase chain reaction (PCR) are now widely available. NAAT has been estimated to have a sensitivity of over 90% and a specificity of 99%, regardless of sampling from a cervical swab or by urine specimen.<sup>28</sup> A screen for *C. trachomatis* infection is recommended before women undergo any form of uterine instrumentation.

## Rationale for this study

Miscarriage affects one in five pregnancies and little progress has been made in understanding and treating this distressing condition.<sup>1</sup> *C. trachomatis* is the most common sexually transmitted infection in the UK.<sup>2</sup> *C. trachomatis* infection can have serious health consequences, including fallopian tube damage. Untreated *C. trachomatis* infection and tubal damage have been associated with miscarriage and adverse pregnancy outcomes such as preterm birth, low birth weight and stillbirth.<sup>4-6</sup> A cohort study is needed to establish the prevalence of tubal disease in women with recurrent miscarriages. HyCoSy will be performed to identify tubal disease and establish the magnitude of the problem in the

recurrent miscarriage population. The prognosis of tubal disease on miscarriage and other obstetric outcomes, and the role of medical interventions such as tubal surgery (to treat hydrosalpinx) on reducing miscarriage and adverse obstetric outcomes will also be studied.

## Objectives

1. Establish the prevalence of hydrosalpinx in the recurrent miscarriage population.
2. Establish the prognosis of women diagnosed with recurrent miscarriage with concurrent hydrosalpinx.
3. Explore the role of tubal surgery for improving reproductive outcomes in women with recurrent miscarriage population and hydrosalpinx.

## Study Design

This is a prospective observational cohort study.

## Population to be studied

Potential participants will be approached at the Tommy's recurrent miscarriage clinic held at the Birmingham Women's Hospital.

### Inclusion criteria

- Age 18-45
- Women diagnosed with recurrent miscarriage i.e. 2 or more miscarriages
- Ability to give informed consent

Such a wide inclusion criteria will enable a true assessment of the prevalence of hydrosalpinges in the recurrent miscarriage population.

### Exclusion criteria

- Allergy to contrast media used for Hysterosalpingo-contrast-sonogram
- Allergy to sonographic gel used for ultrasound scanning
- Anatomical anomaly meaning transvaginal ultrasound scan not possible
- Stenosed/occluded cervix meaning contrast media unable to be introduced via cervix
- Inability to give informed consent
- Pregnant at the time of recruitment
- Declined recruitment

### Identifying Participants

Patients will have been given the patient information leaflet prior to their appointment, when they are sent their appointment details letter.

All women presenting to the clinic will be screened for the eligibility criteria by the routine healthcare provider. They will then be approached by Dr Jayasish Ghosh or one of the members of the research team and offered recruitment into the study.

## Data to be collected and tests to be performed

- Genital infection screen – This is required as per national guidance prior to any uterine instrumentation to reduce the risk of subsequent pelvic inflammatory disease during

Hysterosalpingo-contrast-sonography. The self-taken vulvovaginal swab and one high vaginal swab will then be sent to the microbiology laboratory of the Birmingham Women's Pathology department and will undergo testing for active infection causing bacteria for example *C. Trachomatis*, *N. Gonorrhoea*, *C. Albicans* or bacterial vaginosis. These tests will be in the form of microscopy, culture and sensitivities, polymerase chain reaction and Nugent's scoring. The storage and testing these swabs will be managed in line the Human Tissue Act, 2004.

- To establish the first objective of prevalence, recruited patients will be screened with a Hysterosalpingo-contrast-sonogram (HyCoSy) for the presence or absence of hydrosalpinges.

## Outcomes to be measured

- To establish the second objective of prognosis of women with recurrent miscarriage and concurrent hydrosalpinges, pregnancy outcome data will be collected from both groups of patients (patients who screen positive and those who screen negative for hydrosalpinges). Specifically this will include:
  1. Biochemical pregnancy – a positive pregnancy test either on urine or serum pregnancy testing before ultrasound evidence of a gestational sac
  2. Clinical pregnancy – a pregnancy confirmed by evidence of a fetal heartbeat on ultrasound scan or using ultrasound parameters (ultrasound visualization of a gestational sac, embryonic pole with heartbeat).
  3. Pregnancy loss and at what gestation the pregnancy was lost – if unfortunately the patient suffered from a further miscarriage or still birth, the gestation in weeks of the loss will be noted
  4. Ectopic pregnancy – if a clinical pregnancy is seen on ultrasound scan but its location of implantation has taken place outside the uterine cavity, this is known as an ectopic pregnancy. Ectopic pregnancies are usually inside the fallopian tube although rarely they can be abdominal, ovarian or cervical.
  5. Live births – if fortunately a patient has a live birth this will be noted.
- To explore the role of tubal surgery, the same pregnancy outcomes listed above will be collected for the group of patients that were treated by their usual healthcare provider with surgery for their hydrosalpinges.

## Method of collection of data and outcomes

- The genital infection screen will be performed by taking a self-taken vulvovaginal swab and a high vaginal swab after written informed consent has been gained. The high vaginal swab will be taken by Dr Jayasish Ghosh, a General Medical Council and Royal College of Obstetricians and Gynaecologists registered registrar who is fully competent in such a procedure.
- The presence or absence of hydrosalpinges will be noted at HyCoSy which will be performed after recruitment and written informed consent has been gained. A urine pregnancy test will also be performed prior to the HyCoSy to ensure the patient is not unknowingly already pregnant which would exclude them from the study. The HyCoSy and pregnancy test will be performed by Dr Jayasish Ghosh, a General Medical Council and Royal College of Obstetricians and Gynaecologists registered registrar who is fully competent in such a procedure.
- The pregnancy outcome data listed above will be collected via a telephone consultation or email correspondence, conducted at 6 months and 12 months either post HyCoSy or post

treatment. Should the patient not recall all of the information needed, she will be consented to examine her hospital patient records. Should the patient have had treatment at a different hospital trust, she would also have been consented to collect the specific information needed directly from that particular hospital's patient records.

## Consent to participation and feedback of results

- The conduct of this study will be in accordance with the principles of Good Clinical Practice. All potential participants will be offered a patient information leaflet as well as a copy of the consent form. Participants will be made aware that they can withdraw from the trial at any stage and have their information destroyed.
- Patients will be informed of the results of their genital infection screen and appropriate treatment will be provided as necessary as per Birmingham Women's Hospital antimicrobial prescribing guidelines.
- Patients will also be informed if their pregnancy test performed prior to HyCoSy is positive as they will no longer be able to take part in the study.
- HyCoSy findings will also be shared with the participants at the time of the scan. Any HyCoSy findings that may require urgent gynaecological attention will be referred to the on call gynaecology team at the Birmingham Women's Hospital with the patient's consent. Any other ultrasound findings that require non urgent follow up, will be shared with the GP or routine healthcare provider, with the patient's consent.

## Dissemination of study results

The results of the study in terms of the prevalence of hydrosalpinges in the recurrent miscarriage population and the prognosis of women with recurrent miscarriage and concurrent hydrosalpinx will be disseminated to all participants via an email newsletter at the conclusion of the study. The results of comparing outcomes between patients who were treated for their hydrosalpinges versus those who were not, will be also be disseminated to all study participants and those who were not treated will be offered discussion about treatment after the conclusion of the study if treatment shows significant benefit.

## Withdrawal process

Participants will be informed that they are able to withdraw from the study at any point without giving a reason and without any impact on their care, however, information gathered up until that point may be still used in the study.

## Patient Journey

The patient will be sent the patient information leaflet at the same time as they receive the appointment letter thus ensuring sufficient time to read the written information related to the study before consent is taken. Written informed consent will be taken by good clinical practice trained research midwives or Dr Jayasish Ghosh, a General Medical Council and Royal College of Obstetricians and Gynaecologists registered registrar who is fully competent at taking consent and is also good clinical practice trained.

If a patient consents to participation, they will have the swabs performed as detailed in the method of collection of data and outcomes section above. If the swabs show an infection this will be treated

as per the Birmingham Women's Hospital microbiology prescribing guidelines. At a subsequent appointment they will have a HyCoSy to detect the hydrosalpinges instead of their usual transvaginal pelvic ultrasound scan. A HyCoSy, as detailed in the background section above, incorporates a transvaginal ultrasound scan but is more detailed due to the use of contrast media to detect tubal and uterine pathology which may not be visible on normal transvaginal ultrasound. The result of which will be fed back to the patient and their usual clinician. They will then discuss with this clinician any treatment that maybe required.

If a hydrosalpinx is detected they will discuss with their usual clinician whether tubal surgery and what type would be appropriate. Surgery would then be carried out in the usual manner by the usual clinician. All patients would now be in one of three groups, those with no hydrosalpinx, those with a hydrosalpinx who did not have surgical treatment and those with a hydrosalpinx who did have surgical treatment of their hydrosalpinx. All three cohorts will then be followed up for 12 months as detailed in the method of collection of data and outcome section above. A pictorial representation of the patient journey is shown below in the study flow chart section.

## Accrual and Analysis

### Sample Size

We will attempt to recruit approximately 203 patients. For a prevalence survey in a condition where the prevalence is unknown, but it is not expected to be higher than 25% for obtaining a 95% level of confidence and precision at 0.05 we have estimated that 184 patients are required. To account for up to a 10% level of attrition, 203 patients should be recruited.

### Statistical Analysis

For the prevalence survey of hydrosalpinx in the recurrent miscarriage population data will be presented using descriptive statistics. We will examine the prevalence data within subgroups created from clinical characteristics for example age, body mass index, previous sexually transmitted infections to identify high risk groups for hydrosalpinx.

For the comparison of the cohorts we will report numbers of individuals at each stage of study e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. The reasons for non-participation at each stage will be described. We will also describe the characteristics of study participants (e.g. demographic, clinical) and potential confounders (e.g. age, body mass index.) We will indicate the number of participants with missing data for each variable of interest and compute data for missing covariates using maximum likelihood estimation. We will summarise follow-up time (e.g. average and total amount) and report numbers of outcome events over time for each cohort. We will report unadjusted relative risk estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. at the 95% confidence interval). We will endeavour to translate estimates of relative risk into absolute risk for a meaningful time period. Subgroup analyses will be also performed according to the risk stratification described above.

## Data management and archiving

The swab results and ultrasound records will be kept in locked surroundings at all times at the Birmingham Women's Hospital NHS Foundation Trust. A separate log will be kept which connects the patient identifiable data with the study allocation number and this will be destroyed at the end of the study period. Information will be collected on secured computers and all research team members will have had prior training in Good Clinical Practice.

All data originating from this research will be kept in secured, locked surroundings in the Academic Department of Birmingham Women's Hospital. Electronic data will be held on the University of Birmingham Network and it will be accessed through University of Birmingham computers. All results, records and data will be managed in line with the Data Protection Act, 1998 and the Human Tissue Act, 2004.

## Safety reporting (Adverse Events and Serious Adverse Events)

Adverse events (AE) are defined as any untoward medical occurrence in a study participant, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease. Occurrence of AEs will be recorded and reported to chief investigator. Any SAE will also be recorded in the study notes.

When an AE has occurred which is suspected to be a potential SAE, the Chief Investigator will assess the causality and severity of the AE. If the AE is confirmed as being a SAE, the CI will follow the University of Birmingham Standard Operating Procedures for SAE reporting, including reporting to the Sponsor. Follow-up of the event will continue until the event is considered resolved by all parties involved or a final outcome has been reached.

## Serious breaches

In the unlikely event that a Serious Breach occurs, standard University of Birmingham procedures and SOPs for Serious Breach reporting will be followed.

## Insurance and indemnity

The University of Birmingham purchases Clinical Trials Cover, which provides automatic cover for all Clinical Trials including those where the University accepts responsibility as sponsor, for any harm that comes about due to the University's, or its staff's negligence in relation to the design or management of the trial. In some cases, cover can also be provided in the case of non-negligent harm to participants.

## End of study definition

Within 90 days of the final endpoint assessment of the final participant the main REC will be notified of the study's end and a summary of the clinical study report will be provided within 12 months of the end of the study. The study is expected to last three years.

## Study Oversight and Quality Assurance processes

The Chief Investigator will oversee the quality of the study, and the study team will allow representatives of the Sponsor to monitor the study, including access to source documents as requested.

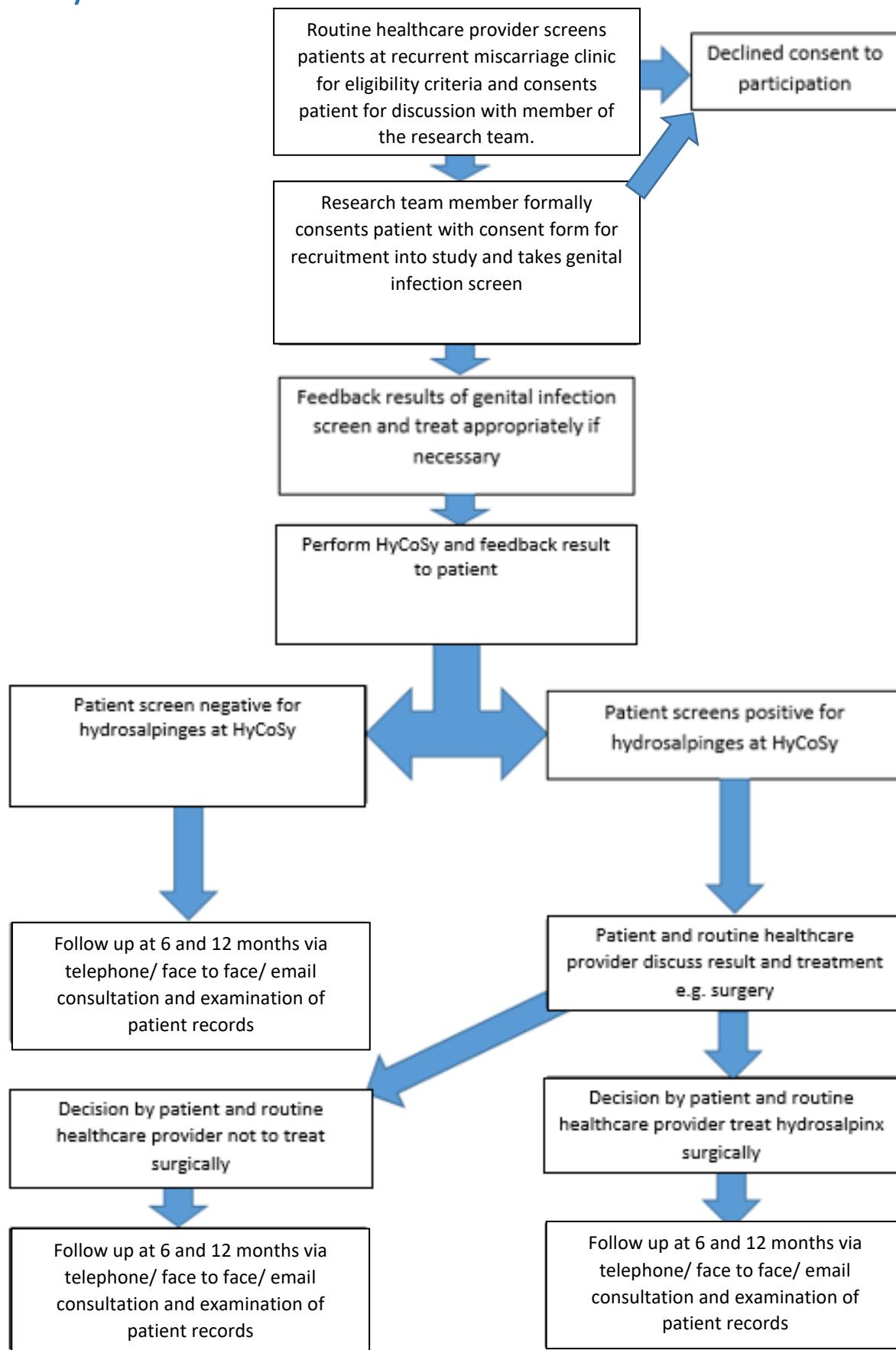
## Funding and Sponsorship

Study funding is provided by Tommy's a UK charity that funds research into miscarriage, stillbirth and premature birth. *Tommy's National Centre for Miscarriage Research* is directed from The University

of Birmingham by Professor Arri Coomarasamy but jointly hosted by three other UK universities, namely Imperial College London and The University of Warwick and is based at four NHS hospitals in Birmingham, Coventry and London. The partner institutions work closely with the University of Birmingham to ensure synergy and added value, whilst a vibrant programme of joint research meetings enables junior and senior colleagues to share disciplinary expertise and research news. The centre is seeking to understand why miscarriage happens, if it is likely to happen again, how to prevent it, and how to provide appropriate aftercare.

The University of Birmingham will be sponsoring this study (sponsorship reference number RG\_17-150). *The University of Birmingham* is characterised by a tradition of innovation. Research at the University has broken new ground, pushed forward the boundaries of knowledge and made an impact on people's lives. The University was ranked 15<sup>th</sup> in the UK and 82<sup>nd</sup> in the world in the QS World University Rankings for 2016-17.

## Study Flow Chart



## List of References

1. Miscarriage Association. *Background information, Miscarriage*. Available from: <https://www.miscarriageassociation.org.uk/media-queries/background-information/> [Accessed July 13<sup>th</sup> 2017]
2. Public Health England. *Sexually Transmitted Infections and Chlamydia Screening in England, 2016*. 2017. Available from: [https://www.gov.uk/government/uploads/system/uploads/attachment\\_data/file/617020/Health\\_Protection\\_Report\\_STIs\\_NCSP\\_2017.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/617020/Health_Protection_Report_STIs_NCSP_2017.pdf) [Accessed: July 13th 2017]
3. Mamoun Kassabji, Jerry A. Sims, Lisa Butler, Suheil J. Muasher Reduced pregnancy outcome in patients with unilateral or bilateral hydrosalpinx after in vitro fertilization. *European Journal of Obstetrics & Gynecology and Reproductive Biology*. 1994; 56(2): 129-132. Available from: <http://www.sciencedirect.com/science/article/pii/0028224394902690> [Accessed July 13<sup>th</sup> 2017]
4. Giakoumelou S, Wheelhouse N, Cuschieri K, Entrican G, Howie SE, Horne AW. The role of infection in miscarriage. *Hum Reprod Update*. 2016; 22(1): 116-33. doi:10.1093/humupd/dmv041.
5. Gravett MG, Nelson HP, DeRouen T, Critchlow C, Eschenbach DA, Holmes KK. Independent Associations of Bacterial Vaginosis and Chlamydia trachomatis Infection With Adverse Pregnancy Outcome. *JAMA*.1986; 256(14):1899-1903. doi:10.1001/jama.1986.03380140069024
6. Martin DH, Koutsky L, Eschenbach DA, Daling JR, Alexander ER, Benedetti JK, Holmes KK. Prematurity and Perinatal Mortality in Pregnancies Complicated by Maternal Chlamydia trachomatis Infections. *JAMA*.1982; 247(11):1585-1588. doi:10.1001/jama.1982.03320360035027
7. NHS Digital. *Hospital Maternity Activity 2015-2016*. 2016. Available from: <http://content.digital.nhs.uk/searchcatalogue?productid=23494&q=%22nhs+maternity+statistics%22&topics=0%2fHospital+care&sort=Relevance&size=10&page=1#top> [Accessed July 13<sup>th</sup> 2017]
8. Ingrid H. Lok, Richard Neugebauer, Psychological morbidity following miscarriage. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2007; 21(2): 229-247. doi:10.1016/j.bpobgyn.2006.11.007
9. M. Goddijn, N.J. Leschot. Genetic aspects of miscarriage. *Best Practice & Research Clinical Obstetrics & Gynaecology*. 2000; 14(5): 855-865. Available from: <http://dx.doi.org/10.1053/beog.2000.0124>. [Accessed July 13<sup>th</sup> 2017]
10. de Braekeleer M, Dao TN. Cytogenetic studies in couples experiencing repeated pregnancy losses. *Hum Reprod*. 1990; 5(5): 519–28.
11. Clifford K, Rai R, Watson H, Regan L. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. *Hum Reprod*. 1994; 9(7) :1328–32.
12. Mary D. Stephenson, Sony Sierra. Reproductive outcomes in recurrent pregnancy loss associated with a parental carrier of a structural chromosome rearrangement. *Hum Reprod*. 2006; 21(4): 1076-1082. doi: 10.1093/humrep/dei417

13. Franssen MTM, Korevaar JC, van der Veen F, Leschot NJ, Bossuyt PMM, Goddijn M. Reproductive outcome after chromosome analysis in couples with two or more miscarriages: case-control study. *British Medical Journal*. 2006; 332(7544): 759-763. doi:10.1136/bmj.38735.459144.2F.
14. Carp, Howard et al. Karyotype of the abortus in recurrent miscarriage. *Fertility and Sterility*. 2001; 75(4): 678 – 682. Available from: [http://dx.doi.org/10.1016/S0015-0282\(00\)01801-X](http://dx.doi.org/10.1016/S0015-0282(00)01801-X) [Accessed July 13<sup>th</sup> 2017]
15. M.D. Stephenson, K.A. Awartani, W.P. Robinson. Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case-control study. *Hum Reprod*. 2002; 17(2): 446-451. doi: 10.1093/humrep/17.2.446
16. Grigoris F. Grimbizis, Michel Camus, Basil C. Tarlatzis, John N. Bontis, Paul Devroey. Clinical implications of uterine malformations and hysteroscopic treatment results. *Hum Reprod Update*. 2001; 7(2): 161-174. doi: 10.1093/humupd/7.2.161
17. R. Salim, L. Regan, B. Woelfer, M. Backos, D. Jurkovic. A comparative study of the morphology of congenital uterine anomalies in women with and without a history of recurrent first trimester miscarriage. *Hum Reprod*. 2003; 18(1): 162-166. doi: 10.1093/humrep/deg030
18. U Hanson et al. Relationship between Haemoglobin A1C in early type 1 (insulin-dependent) diabetic pregnancy and the occurrence of spontaneous abortion and fetal malformation in Sweden. *Diabetologia*. 1990; 33(2): 100-104.
19. R.S Rai, K. Clifford, H. Cohen, L. Regan; High prospective fetal loss rate in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. *Hum Reprod*. 1995; 10(12): 3301-3304. doi: 10.1093/oxfordjournals.humrep.a135907
20. Regan L, Backos M, Rai R. *The Investigation and Treatment of Couples with Recurrent First trimester and Second-trimester Miscarriage*. Royal College of Obstetricians and Gynaecologists. Green-top Guideline No. 17. April 2011. Available from: [https://www.rcog.org.uk/globalassets/documents/guidelines/gtg\\_17.pdf](https://www.rcog.org.uk/globalassets/documents/guidelines/gtg_17.pdf) [Accessed July 13<sup>th</sup> 2017]
21. Willard Cates, Judith N. Wasserheit. Genital chlamydial infections: Epidemiology and reproductive sequelae. *American Journal of Obstetrics and Gynecology*. 1991; 164(6): 1771-1781. Available from: <http://www.sciencedirect.com/science/article/pii/000293789190559A?showall%3Dtrue%26via%3Dihub> [Accessed July 13<sup>th</sup> 2017]
22. Hulusi B Zeyneloglu, Aydin Arici, David L Olive. Adverse effects of hydrosalpinx on pregnancy rates after in vitro fertilization-embryo transfer. *Fertility and Sterility*. 1998; 70(3): 492-499.
23. National Institute for Health and Care Excellence. *Fertility problems: assessment and treatment for people with fertility problems*. NICE Clinical guideline No. 156. February 2013. Available from: <https://www.nice.org.uk/guidance/cg156/evidence/full-guideline-pdf-188539453> [Accessed July 13<sup>th</sup> 2017]
24. A Strandell, A Sjögren, U Bentin-Ley, J Thorburn, L Hamberger, M Brännström. Hydrosalpinx fluid does not adversely affect the normal development of human embryos and implantation in vitro. *Hum Reprod*. 1998; 13(10): 2921-2925. doi: 10.1093/humrep/13.10.2921
25. Mohamed A. Bedaiwy, Jeffrey M. Goldberg, Tommaso Falcone, Mamta Singh, David Nelson, Hamdy Azab, Xia Wang, Rakesh Sharma. Relationship between oxidative stress and

- embryotoxicity of hydrosalpingeal fluid. *Hum Reprod* 2002; 17(3): 601-604. doi: 10.1093/humrep/17.3.60
26. Sung, L., Copperman, A.B., Goldstein, M., Lapinski, R., Bustillo, M. and Mukherjee, T. Characterization of cytokines (IL-1 $\alpha$ , IL-1 $\beta$ , IL-8, and TNF- $\alpha$ ) in human hydrosalpinx fluid: a potential mechanism for embryo toxicity (abstract). *Fertil. Steril.* 1997; 1001(1997): S78
  27. Mansour RT, Aboulghar MA, Serour GI, Riad R. Fluid accumulation of the uterine cavity before embryo transfer: a possible hindrance for implantation. *Journal of Assisted Reproduction and Genetics*. 1991; 8(3): 157-159.
  28. J. R. Papp, J. Schachter, C. A. Gaydos, B. Van Der Pol. *Recommendations for the Laboratory-Based Detection of Chlamydia trachomatis and Neisseria gonorrhoeae*. Centres for Disease Control and Prevention. 63(RR02). March 2014. Available from <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6302a1.htm> [Accessed July 13<sup>th</sup> 2017]