Impact of DNA fragmentation in sperm on the pregnancy outcome after intra-uterine insemination in a spontaneous cycle

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Introduction

Infertility affects about 10% of all couples and is defined by a failure to achieve a clinical pregnancy within a year of regular unprotected sexual intercourse. (Boivin 2007, Zegers-Hochschild 2009).

Male infertility accounts for nearly 30% of diagnoses, whilst an estimated 40% of male factor infertility remains unexplained (de Kretser 1997) and most of male factor infertility cannot be treated adequately.

The current diagnosis of male infertility is based on semen criteria updated by the WHO in 2010. These guidelines would consider a man normal with reference values of greater than 15 million sperm, greater than 4% normal morphology, and 32% progressive motility. By doing so more men are considered fertile while there may be an unnoticed rise in the number of infertile men meantime being assigned to the 'unexplained' category. Furthermore , although the WHO classification suggests accuracy through a methodology harmonized across laboratories (Wang 2014), the relevance for the choice of treatment and the predictive value for an infertile couple embarking on medically assisted reproduction technologies (MAR) is questionable. (Hamilton 2015)

In other words, there is a strong clinical need to distinguish fertile from infertile men through new sperm function testing and to be able to select both the patient population who will benefit from MAR as well as the type of treatment.

Numerous studies utilizing different techniques for assessing sperm DNA fragmentation support the existence of a significant association between sperm DNA damage and pregnancy outcomes (Collins et al., 2008). Moreover, a significant number of subfertile men have abnormal DNA integrity despite normal semen parameters (Kodama et al., 1997; Spano et al., 2000; Zini et al., 2001). The level of DNA fragmentation correlates negatively with pregnancy and delivery in both natural and assisted conceptions (Evenson & Wixon 2006; Zhang et al. 2008; Zini 2011).

Our laboratory has set up a program where direct DNA fragmentation testing with terminal deoxyuridine nick end labeling (TUNEL) assay (Mitchell 2011) on fresh samples was established both on the total and vital fraction, before and after density gradient. The data results have been submitted and will be available soon (Punjabi 2017).

In this prospective cohort study we aim to investigate the role of sperm DNA fragmentation analysis in selecting the patient who will benefit from intra-uterine insemination (IUI) therapy since IUI is still considered the first step in MAR and is performed at a large scale in Belgium and worldwide.

The overall success rate of IUI remains controversial. On average, reported clinical pregnancy rates are only 5–13% per IUI cycle (Bensdorp et al., 2007; Goverde et al., 2000; Guzick et al., 1999; Nyboe Andersen et al., 2009; Steures et al., 2004, 2007; The ESHRE Capri Workshop Group, 2009; Tummon et al., 1997; Verhulst et al., 2006).

A Cochrane analysis pointed to the position of IUI in male subfertility treatment as being one of the most frequently used treatments (Veltman-Verhulst et al., 2016) although its efficacy in the setting of mild male/unexplained infertility is yet to be proven. The authors concluded that there was insufficient evidence of effectiveness to recommend or advise against IUI.

In addition, the literature pertaining IUI consists of a broad range of indications for therapy, ranging from but not limited to: unexplained fertility, mild male infertility and mild endometriosis. Respectively, each of these diagnoses also have slightly different definitions from one study to another, thus making a univocal recommendation concerning IUI very challenging.

Our goal would be to better select patients for IUI, widely considered less invasive and expensive therapy than others, as about 50% of the patients entering an IUI program will not become pregnant after 4-6 IUI cycles and thus in selected cases (Van Hoof et al., 2009) progression to more advanced fertility therapy could be beneficial.

Study Objectives

The objective is to test the hypothesis that DNA fragmentation testing can play a diagnostic and thus pivotal role in selecting the patient who will benefit from intra-uterine insemination (IUI) therapy leading to a higher clinical pregnancy and live birth rate (primary outcomes) compared to the standard semen criteria.

Additionally, DNA fragmentation will be measured both at the time of the diagnostic work-up as at the time of insemination with evaluation in the neat and washed specimen, both in the total and vital fraction. We aim to assess whether density gradient retrieves high quality motile sperm with little DNA damage and where DNA fragmentation testing best fits in the clinical patient pathway (secondary outcomes).

Study Endpoints

Primary outcomes:

- DNA fragmentation as a predictor of clinical pregnancy and live birth rate

Secondary outcomes:

- DNA fragmentation in the total and vital fraction before and after density gradient in the diagnostic sample (pre-IUI)
- DNA fragmentation in the total and vital fraction before and after density gradient in the therapeutic sample (IUI sample)
- DNA fragmentation in relation to the cumulative clinical pregnancy and live birth rate

Study Phase & Design

Prospective interventional study
Duration: 24 months

Source population: couples with unexplained, mild male infertility, mild endometriosis defined by revised American Society for Reproductive Medicine stage I and II

Single center, university setting, no commercial affiliation

Study Population

Inclusion criteria:

Couples seeking fertility treatment after at least 12 months of unprotected intercourse are eligible. All couples underwent basic fertility investigations which included semen analysis, evaluation of menstrual cycle, and tubal patency testing (Chlamydia antibody test, hysterosalpingography/HyfoSy or laparoscopy).

All participating couples should provide written informed consent

Inclusion criteria are

- Unexplained infertility
    - We classify couples as having unexplained subfertility when the fertility investigations showed at least one patent fallopian tube, a normal ovulatory

menstrual cycle (26-32), and a normal semen analysis (pre-wash total motile sperm count above 10 million).

- Mild male infertility defined as one or more abnormal semenparameters according to the WHO 2010 criteria on at least two examinations; diagnosis is determined by most abnormal result
- Women: age between 18 and 40 years, BMI between 18,5-30, presence of at least one patent tube on hysterosalpingography/Hysterosalpingo foam sonography (Hyfosy) and/or laparoscopy, normal uterine cavity (ultrasound, hysterosalpingography or laparoscopy), regular menstrual cycle (24-38 days, FIGO recommendations 2011)
- Men: age between 18 and 65 years, BMI between 18,5-30, normal semen analysis or mild male subfertility

- Exclusion criteria: double sided tubal disease, severe endometriosis (classified as revised American Society for Reproductive Medicine stage III or IV), premature ovarian failure, and known endocrine disorders (such as Cushing's syndrome or adrenal hyperplasia), azoö- or necrozoöspermia

Statistics

- Sample size: 120 patients
  A power analysis was performed to estimate the sample size needed for ROC curve analysis. Since there is scarce literature for sperm DNA fragmentation on an IUI sample both before and after gradient, we cannot rely on reference values to estimate the area under the curve (AUC) or distribution of cases versus controls (kappa). In function of a significance of 0,05 (type I error) and power of 0,8 (1-type II error), a kappa varying between 1:1 and 1:3 and AUC varying between 0,7-0,75 we estimate a minimum of 38 patients and a maximum of 130 patients should be included.

- Diagnostic parameters: sensitivity, specificity, area under receiver operator curve, predictive value, likelihood ratio, odds ratio
- Null hypothesis:
  1) There is no correlation between DNA fragmentation and clinical pregnancy/live birth after IUI
  2) Density gradient isn't capable of selecting motile sperm with low DNA fragmentation for insemination

Clinical protocol

- IUI in a spontaneous cycle
- Basal ultrasound and hormonal analysis on cycle day 2 or 3 (estradiol (E2), progesterone (P), luteinizing hormone (LH) as well as follicle stimulating hormone (FSH))
- Cycle monitoring from D10-12 with ultrasound and/or hormonal analysis (E2, P, LH)
- 250 microgram hCG or 5000 IU is administered to induce ovulation when a maximum of 2 dominant follicles are present. Either Pregnyl, Choragon, Ovitrelle will be used
- IUI is planned between 32-40 hours after HCG administration or ± 24-26 hours after detection of spontaneous LH surge
- Both ultrasound and hormonal analysis (E2, P, LH) are done on the day of planning of IUI (day of hCG administration or day of LH surge)
- Sperm preparation is performed according to local validated procedures in accordance with the 2010 WHO criteria. DNA fragmentation analysis by TUNEL assay will be carried out both before and after density gradient.
- Patients are advised to rest in the supine position during 15 minutes immediately after IUI according to evidence from a RCT (Custers et al. 2009)

- Detection of pregnancy
  - βHCG testing is performed 15-20 days after insemination
  - A second βHCG testing is performed 7 days later to confirm a positive evolution of pregnancy
  - In case of a positive evolution of βHCG an ultrasound is performed around 5-6 weeks after IUI
  - Follow-up of pregnancy until birth through staff, telephone or e-mail contact until birth

- Items to be registered
  - Number of eligible patients (patients who fulfilled inclusion criteria and to whom the study was proposed)
  - Number of drop outs between inclusion and end of treatment
  - Number of spontaneous pregnancies between inclusion and end of treatment
  - Number of live births between inclusion and end of treatment

  - Hunault score and modified Hunault-score according to Bensdorp et al.
  - All Belrap items
  - Semen analysis methodology checklist according to Björndahl et al. (Appendix)
  - Patient population characteristics:
    - Infertility: duration, primary versus secondary, female/male/mixed/unexplained
    - Cause of female infertility: Ovulatory disorder, Anatomical infertility, Endometriosis (revised American Society for Reproductive Medicine classifcation) or Adenomyosis
    - Possible cause of male infertility: Varicocoele, Cryptorchidism, Male accessory gland infections, Germ cell malignancies and testicular microcalcifications, Disorders of ejaculation, Idiopathic. Primary spermatic failure, genetic disorders of infertility, obstructive azoöspermia, hypogonadism were not reported since azoöspermia is considered an exclusion criterium.

- Data analysis and Publication policy
  Data analysis and preparation for publication will be done at the University Hospital of Antwerp / University of Antwerp.

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