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**LIPIODOL FERTILITY ENHANCEMENT
IN
UNEXPLAINED AND ENDOMETRIOSIS-RELATED
INFERTILITY**

NEIL PHILIP JOHNSON

**LIPIODOL FERTILITY ENHANCEMENT
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INFERTILITY**

ASSOCIATE PROFESSOR NEIL PHILIP JOHNSON

**A thesis submitted for the degree Doctor of Medicine
University of Auckland
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DEDICATION

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PUBLICATIONS ARISING FROM THIS THESIS

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SUBMITTED PAPERS ARISING FROM THIS THESIS

Johnson NP, Kwok R, Stewart AW, Saththianathan M, Hadden WE, Chamley LW. Lipiodol fertility enhancement: two year follow up of a randomised trial suggests a transient benefit in endometriosis but a sustained benefit in unexplained infertility. *Hum Reprod*, submitted 2007.

AWARDS ARISING FROM RESEARCH IN THIS THESIS

2005: The Shan S Ratnam 'Young Gynaecologist' Award for demonstrating leadership in research at the 19th Asia Oceania Congress of Obstetrics & Gynaecology (Seoul, Korea).

2005: Award for the Best Free Communication at the Australian Gynaecological Endoscopy Society 15th Annual Scientific Meeting (Perth, Australia) for 'Poppy seed oil or surgery: is lipiodol a more effective fertility treatment than laparoscopic endometriosis surgery?'

2004: New Zealand Committee RANZCOG Young Gynaecologist Award for outstanding contributions to gynaecology research in New Zealand.

2003: Prize for Best Clinical Paper by a Young Clinician at Fertility Society of Australia ASM (Perth, Australia) for 'The FLUSH Trial – RCT of lipiodol flushing'.

2003: Prize for the Best Presentation at the RANZCOG ASM (Auckland) for 'The FLUSH Trial – a randomised trial of lipiodol flushing for unexplained subfertility by hysterosalpingography'.

ABBREVIATIONS USED IN THIS THESIS

AIH	Artificial insemination by husband
ART	Assisted reproductive technology
CC	Clomiphene citrate
CD	Cluster determinant
CI	Confidence interval
CSF-1	Colony stimulating factor-1
DC	Dendritic cell
df	Degrees of freedom
DNA	Deoxyribonucleic acid
FLUSH	Flushing with lipiodol for unexplained subfertility by hysterosalpingography
FSH	Follicle stimulating hormone
g	Grams
HSG	Hysterosalpingogram
ICSI	Intracytoplasmic sperm injection
Ig	Immunoglobulin
IGFBP-1	Insulin-like growth factor 1 binding protein
IL-6	Interleukin-6
IQR	Inter-quartile range
IUI	Intrauterine insemination
IVF	In vitro fertilisation
LH	Luteinising hormone
MHC	Major histocompatibility complex
ml	Millilitre
mmol	Millimoles
NK	Natural killer
NNT	Number needed to treat
NRR	National Research Register
OR	Odds ratio
OSCM	Oil soluble contrast medium
p	Probability value
PBS	Phosphate buffered saline
pmol	Picomoles
r-ASRM	Revised American Society of Reproductive Medicine
RP	Relative probability
RR	Relative risk
TNF- α	Tumour necrotising factor-alpha
RevMan	Review Manager computer software
RCT	Randomised controlled trial
sd	Standard deviation
VAS	Visual analogue scale
WHO	World Health Organisation
WMD	Weighted mean difference
WSCM	Water soluble contrast medium

SYNOPSIS

This thesis sought to investigate the 'old clinician's tale' that having the fallopian tubes flushed in a tubal patency test (with lipiodol, an oil soluble contrast medium) improves a woman's fertility. It focused upon (a) pre-existing evidence; (b) mechanisms of effect of lipiodol; (c) generating further evidence through a definitive randomised controlled trial; (d) adoption of lipiodol treatment into clinical practice.

EXECUTIVE ABSTRACT

Objectives

This thesis had the following objectives:

- 1) To assess the existing evidence base for the effectiveness of tubal flushing as a treatment for infertility (Section II, Chapters 4 and 5).
- 2) To assess current practice and prior beliefs amongst Australasian fertility specialists concerning the role of tubal flushing as a treatment for infertility (Section III, Chapter 6).
- 3) To investigate the possible mechanism of the fertility enhancing effect of the oil soluble contrast medium lipiodol, and specifically whether there is an effect on the endometrium (Section IV, Chapter 7).
- 4) To generate definitive evidence from a randomised controlled trial for the effectiveness of lipiodol flushing as a treatment for infertility (Section V, Chapters 8 and 9).
- 5) To evaluate the adoption of lipiodol flushing as an innovative treatment into clinical practice (Section VI, Chapter 10).

Methods

The work undertaken in this thesis was based on prospective study protocols using the following research methodologies:

- Systematic reviews and meta-analyses of treatment efficacy to meet objective 1.
- Two types of structured survey questionnaires with a Bayesian analysis to meet objective 2.
- A randomised animal study involving 60 Swiss white mice, combined with genital flushing procedures under anaesthesia, some of which involved

microsurgical techniques, followed by genital tissue harvesting, tissue preparation and immunohistochemistry studies to meet objective 3.

- An open, parallel group, single centre, randomised controlled trial involving 158 women with unexplained and endometriosis-related infertility to meet objective 4.
- A survival analysis of women in the lipiodol flushing randomised trial to meet objectives 4 and 5.
- A prospective observational study of the first 100 women to undergo lipiodol flushing in clinical practice to meet objective 5.
- A clinical hysterosalpingogram procedure to meet objective 4 and 5.

Results

- 1) Eight randomised controlled trials involving 1,971 women were identified and included in the systematic review. Tubal flushing with oil soluble contrast media versus no intervention was associated with a significant increase in the odds of pregnancy (Peto odds ratio [OR] 3.57, 95% confidence interval [CI] 1.76 to 7.23) but there were no data for live birth. There were no data from RCTs to assess tubal flushing with water-soluble media versus no intervention. Tubal flushing with oil soluble contrast media was associated with a significant increase in the odds of live birth versus tubal flushing with water soluble contrast media (OR 1.49, 95% CI 1.05 to 2.11) but the odds of pregnancy showed no significant difference (OR 1.24, 95% CI 0.97 to 1.57) and there was evidence of statistical heterogeneity for these two outcomes. The addition of oil soluble contrast media to flushing with water soluble contrast media (water plus oil soluble contrast media versus water soluble contrast media alone) showed no significant difference in the odds of live birth (OR 1.06, 95% CI 0.64 to 1.77) or pregnancy (OR 1.16, 95% CI 0.78 to 1.70).

- 2) Nineteen Australasian fertility specialists returned survey questionnaires. Eighteen of the 19 specialists believed that lipiodol flushing was more likely to be beneficial than harmful. The most widely held prior belief, reflected in both textual and numerical responses, was that lipiodol was likely to produce a small beneficial response. The credible limits of this belief were compatible with a reasonable fertility benefit, as more than 50% believed that a 1.5-fold increase in pregnancy rate was plausible. The two surveys found that a 1.2-fold or 1.4-fold increase in pregnancy rate was the median expected level of benefit at which clinicians would have been inclined to recommend lipiodol flushing to their patients (combined range 1.1 to 2.3-fold). Individual and collective equipoise was justification to proceed with a definitive randomised controlled trial.
- 3) The mean number of cluster determinant (CD) 205⁺ uterine dendritic cells decreased significantly in mice following lipiodol treatment compared to sham treated and saline treated mice, particularly in endometrial and sub-endometrial tissues. The mean number of CD1⁺ uterine dendritic cells increased significantly following lipiodol treatment compared to sham-treatment. No significant differences were found in the mean number of total leukocytes or macrophages in the murine uterus between the three treatment groups.
- 4) Six month follow up of the randomised trial of 158 women showed that lipiodol flushing resulted in a significant increase in pregnancy (48.0% versus 10.8%, RR 4.44, 95% CI 1.61-12.21) and live birth (40.0% versus 10.8%, RR 3.70, 95% CI 1.30-10.50) rates versus no intervention for women with endometriosis (n=62), although there was no significant difference in pregnancy (33.3% versus 20.8%, RR 1.60, 95% CI 0.81-3.16) or live birth (27.1% versus 14.6%, RR 1.86, 95% CI 0.81-4.25) rates for women with unexplained infertility without confirmed endometriosis (n=96). Survival analysis up to 24 months showed a significant benefit in

overall pregnancy rate following lipiodol treatment (hazard ratio 2.0, 95% confidence interval [CI] 1.3 to 3.2) for the combined endometriosis and unexplained infertility populations. Amongst women with endometriosis, the benefit in pregnancy rate seen in the first 6 months following lipiodol (hazard ratio 5.4, 95% CI 2.1 to 14.2) was not present at 6 to 24 months following lipiodol (hazard ratio 0.6, 95% CI 0.2 to 2.1). There was a more consistent effect of lipiodol on fertility throughout the 24 month follow up amongst women with unexplained infertility (hazard ratio 2.0, 95% CI 1.1 to 3.5).

- 5) Six month follow up in the observational study of 100 women undergoing lipiodol flushing as an innovative treatment in clinical practice showed an overall pregnancy rate 30% and live birth or ongoing pregnancy rate 27% six months after the procedure. For women under 40 years old, a 32% pregnancy rate and 25% live birth or ongoing pregnancy rate was seen in women with unexplained infertility; a 50% pregnancy rate and 47% live birth or ongoing pregnancy rate was seen in women with endometriosis. Of women aged 40 years and older, the pregnancy rate was 13% and the live birth or ongoing pregnancy rate was 13%. The pregnancy rates included those occurring after additional interventions, such as intrauterine insemination and in-vitro fertilisation, accounting for 12 of the 30 pregnancies. There were no treatment complications.

Conclusions

The conclusions of this thesis are as follows.

- Lipiodol flushing is a simple, inexpensive, effective fertility treatment, which carries a very low chance of complications and no increased chance of multiple pregnancy.

- Lipiodol treatment is particularly effective in the short term for women with endometriosis who have normal patent fallopian tubes.
- The fertility benefit from lipiodol treatment lasts longer for women with pure unexplained infertility than for women with endometriosis.
- The level of benefit from lipiodol treatment for women with unexplained and endometriosis-related infertility is of sufficient magnitude to convince most fertility specialists surveyed that it is a worthwhile treatment to offer routinely in clinical practice; however complex factors govern the implementation of an innovative fertility treatment.
- Observational study of the first 100 women to undergo lipiodol treatment in clinical practice has provided further evidence of the efficacy and safety of this approach.
- Uterine dendritic cell changes following lipiodol flushing in mice suggest that the mechanism of the fertility enhancing effect might be an immunobiologic effect on the endometrium that could improve the receptivity of the endometrium (rather than a mechanical tubal flushing effect), although this hypothesis requires further exploration in women.