

Oral Abstracts

Treating HIV Discordant Couples

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Aim: To describe a service to assist HIV positive patients to conceive with minimum risks of transmission, particularly to the children.

Methods: In 1998 a multidisciplinary committee started to develop a protocol. HIV assays on semen were validated. Infection Control in the ART and Andrology laboratories was assessed. The Clinical Ethics Advisory Committee, Board and Insurers of RWH approved the treatment of positive men in 2002 and women in 2006. The male positive female negative (M+F-) protocol involves medical assessment and counselling, confirmation of undetectable viral load in blood and two seminal plasma samples then cryopreservation of 3–6 semen samples each of which is tested for viral load in seminal plasma and where possible proviral DNA in a sperm preparation. Only samples with undetectable results are used for colloidal silica gradient sperm preparation for IUI, IVF or ICSI depending on the couple's choice, predicted fertility or requirement for PGD (eg, for haemophilia). Testing for seroconversion is instituted after treatment and during pregnancy. M–F+ couples are treated by IUI or ART provided the woman has stable undetectable viral loads and agrees to close monitoring during pregnancy.

Results: Treatment of M+F- discordant couples started in 2002: 29 have been seen, 15 have commenced treatment, 3 have stopped not pregnant and 8 have babies or continuing pregnancies. The female partners remain HIV negative. Three M–F+ couples have started treatment in 2006 and there is 1 pregnancy.

Conclusion: Assisting HIV positive patients to conceive with low risk of transmission is successful but labour intensive.

Elevated Glucose Levels Induce Lipid Peroxidation and DNA Damage in Human Spermatozoa

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Aim: Sperm DNA damage has been associated with poor fertilisation rates, embryo development and pregnancy outcome. Lipid peroxidation and sperm DNA damage have been shown to be important factors in the aetiology of defective sperm function. This study was conducted to determine whether or not there is an association between increased levels of glucose concentration, lipid peroxidation and sperm DNA damage.

Method: Sperm samples were obtained from three healthy normospermic donors for this study. Motile sperm were separated from semen samples using density gradient separation. Washed spermatozoa were incubated for 18 hours under 3 different media conditions of varying glucose concentrations (0, 3.15, 30 mM). Lipid peroxidation was assessed using the probe BODIPY (581/591) C₁₁. Sperm DNA damage was determined using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labelling (TUNEL). Differences between sperm parameters were assessed using Student's t-test.

Results: High glucose concentration was associated with a 17% overall increase in lipid peroxidation ($p < 0.01$). Furthermore, incubation in 30 mM glucose also resulted in a significant increase in sperm with DNA damage ($p < 0.05$).

Conclusion: To our knowledge this is the first report providing a possible link between glucose levels in the environment and lipid peroxidation in the human sperm and elevated levels of sperm DNA damage. We hypothesize that these increases may be linked with increased reactive oxygen species. This concept requires further investigation due to its in vivo implications especially for diabetic and obese men where seminal plasma levels of glucose may be elevated.

Pervious Maternal Cyclophosphamide Treatment Affects *in vitro* Fertilization and Causes Chromosomal Abnormalities in Preimplantation Mouse Embryos

(Poster Presentation)

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Aim: Females that are subfertile due to chemotherapy can receive assisted reproduction techniques such as *in vitro* fertilization (IVF), but we must be sure of the embryo health by some means.

Method: Two different groups consist of female NMRI mice, 2–3 and 6–7 weeks of age were injected intraperitoneally with 75 mg cyclophosphamide/kg body weight. Six weeks later, the mice were superovulated. Oocytes were recovered, washed and fertilized with sperm obtained from non-treated male mice, and incubated for 3 days in 5% CO₂ in air. Blastomer's chromosomal status was evaluated by Verlinsk's method.

Results: Cyclophosphamide in both experimental groups, reduced the oocyte fertilization rate and in the 6–7 weeks group fertilization rate was significantly lower compared with control group ($p < 0.01$). Also there was reduction in embryo development in both groups ($p < 0.05$). Aneuploidy increased in the treatment groups compared with control groups, which in the older group was significant ($p < 0.001$).

Conclusion: In regard to the defects in fertilization rate, embryo cleavage, developments and the numerical aberrations in the chromosomes, we suggest pre-implantation genetic diagnosis especially with the Verlinsky's method in order to confirm chromosomal status of the embryos and increasing the pregnancy rate in these patients.

Cryopreservation of Small Numbers of Sperm for Use in Cases with Limited Sperm Retrieval

(Poster Presentation)

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Aim: For men with non-obstructive azoospermia, there is a risk that no sperm or only limited numbers will be found at open testicular biopsy. An ability to cryopreserve and reliably retrieve small numbers of sperm prior to treating the woman would have significant benefits in managing these couples. An *in situ* microdrop method has been evaluated for this purpose.

Methods: In a petri-dish under oil, small numbers (≤ 6) of motile sperm were transferred by micropipette into drops of freezing medium and the dish frozen in LN₂ vapour. Following thawing, the number of sperm recoverable by micropipette was recorded. Post-thaw motility was assessed in further experiments using differing drop sizes (1–10 μ l) and freezing rates (direct placement into LN₂ vapour vs 30 min at 4°C then LN₂ vapour). The outcome from open testicular biopsies was also evaluated.

Results: Of 51 motile sperm frozen, 82% sperm were successfully recovered. However, post-thaw motility was poor with minimal effect of drop size or freezing rate on outcome (post-thaw motilities: LN₂ vapour 3.3%, range 1.6–5.0%; 4°C/LN₂ vapour: 3.1%, range 2.3–4.3%). Of 20 patients having open biopsy, 7 yielded sufficient sperm to freeze using standard methods while one patient had only 2 sperm recovered which were frozen using *in situ* microdrops. No thaws for clinical use have been done to date.

Conclusion: Individually cryopreserved sperm can be reliably recovered using *in situ* microdrops. However, post-thaw motility is limited. Further study is needed to improve post-thaw viability and validate the function of sperm which have lost motility from freezing damage.

Fertility and Reproductive Health in Indigenous Women

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There has been a gradual decline in fertility rates in Australian women. The trend towards delayed childbearing and a decrease in the proportion of women having three or more children have been significant factors in this decline. Fertility rates in Indigenous women remain higher than for all Australian women at 2.15 compared to 1.75 in 2003. Delayed age at childbearing does not appear to be a significant factor in Indigenous women. The median age of Indigenous mothers in 2003, at 24.6 years was 6 years younger than all Australian mothers.

Information on the prevalence of infertility and causes of infertility in Indigenous women is sparse. We do know however that they have a higher prevalence of general health factors such as obesity, smoking and sexually transmitted infections that can affect fertility, general reproductive and metabolic health. Many of these health problems arise in the context of low socioeconomic environments and remote residential location. These factors affect health behaviours, health risk factors and access to health education and health services. These issues are very important in terms of current individual health and contribute to the health of the next generation through their influence on the intrauterine and early childhood environment. This session will explore some of the issues around Indigenous women's fertility and reproductive health particularly in the Top End of the Northern Territory.

Coordination of a Successful Anonymous Altruistic Oocyte Donation Programme

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Aim: To identify the factors behind the management and maintenance of a successful anonymous altruistic oocyte donation programme.

Method: Review of the details of 233 patients having 314 embryo transfers resulting from fresh oocyte donation by 127 donors for the years 2002 to 2006.

Results: 233 oocyte recipients having 314 embryo transfers yielded 125 clinical pregnancies (39.8%/ET, 48.5%/patient). 40 oocyte donors (31%) underwent more than one donation cycle with a maximum of six cycles. Key factors in the recruitment of donors and their retention for more than one donation cycle include: a mix of media advertisement and personal recruitment, management of donors one-on-one by specialist donor coordinators, a high level of support extending beyond formal clinic visits, isolating donors from mainstream patients, minimizing every possible intrusion into their normal lifestyle by the treatment, and positive personalized clinical management of the donors. On-going contact is maintained with donors to ensure that personal contact details are current. This often initiates offers of repeat donation cycles.

Conclusion: Oocyte donation is a complex intrusive process that is attractive to only a small number of highly motivated women. Retaining donors through the screening and donation process requires a personalized specialist approach somewhat different from that used with infertile couples. The success of the programme can be measured by its clinical success and the frequency of repeat donation.

Extended Culture Lives Up To Expectations

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Aim: To determine whether extended culture does select for the embryos most likely to implant without compromising the chances of a patient taking home a baby.

Methods: Monash IVF operates 6 days a week and, on a Saturday, embryos can be transferred on days 2,3,4,5 post insemination. The cumulative pregnancy rates for these days were tracked to assess the efficacy of our extended culture programme.

Patients, <41, had to have at least one embryo transferred and frozen to be included in this study. Patients having an ET on day 2 had their embryos frozen on day 3 and patients having a transfer on day 4 had their embryos frozen on day 5 or 6. Frozen embryos from the stimulated cycles were tracked for transfer and pregnancy to give the cumulative pregnancy rate.

Results:

ET day	Preg rate	FH/embryo Transferred	Additional preg from FET	CumPreg rate	Average Et/Preg
2	70/196 (35%)	32%	36	54%	3.47
3	609/1493 (41%)	30%	304	61%	3.25
4	56/126 (44%)	33%	25	64%	2.5
5	179/366 (49%)	41%	49	62%	2.3

The results show a significant increase in implantation rate between day 5 compared with the other days ($p = 0.020$). There are no significant differences in the cumulative pregnancy rates. The average number of transfers to achieve a pregnancy progressively decreases as the time in culture increases. These differences are significant ($p < 0.01$) between day 5 and 4 compared with days 2 and 3.

Conclusions: For the majority of patients, the results provide compelling data that, extended culture helps achieve the same number of pregnancies, only quicker.

Incidence of Monozygotic Twins is not Related to Duration of Embryo Culture for Both Fresh and Frozen Embryos

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Aim: To investigate the incidence of monozygotic twins (MZT) as related to culture duration for both fresh and frozen embryos.

Introduction: There are conflicting reports as to the incidence of monozygosity in relation to the duration of embryo culture. Reported rates vary from virtually zero to 6% of pregnancies. Clearly the rate of MZT is raised in ART procedures but it is unclear as to the causes.

Methods: Retrospective data analysis of all fresh and frozen embryo transfer cycles with a singleton embryo. This avoids the issue of attempting to identify MZT pregnancies from dizygotic twins. The presence of two fetal hearts was used as evidence for MZT. Results were analysed by χ^2 analysis. Power analysis of the sample size indicated that a 25% difference in the MZT rate would be detected.

Results: The rate of MZT for fresh embryo transfer was 3.0% (10/326) for embryos cultured for 3 days compared with 2.7% (5/187) for 5 days culture. The corresponding figures for frozen embryos were 2.7% (12/437) for day 3 compared with 2.5% (2/79) for day 5. All of these figures are not significantly different.

Conclusion: Neither the length of time in culture nor whether transferred fresh or frozen made any difference to the MZT rate. The increased rate of MZT with ART remains enigmatic.

The Inpatient Birth-Admission Costs of Singleton and Multiple Births after ART Treatment – A Comparison with Spontaneously Conceived Births

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Aim: Despite the trend to transfer fewer embryos during assisted reproductive technology (ART) procedures, multiple births still account for 17% of deliveries in Australia (1). In addition to the risks associated with multiple gestation, recent studies have shown that ART singletons are at risk of poorer perinatal outcomes compared to spontaneously conceived singletons. The aim of this study was to compare the infant and maternal characteristics and inpatient birth-admission costs of ART deliveries to spontaneously conceived deliveries.

Method: A cohort of 5005 mothers who gave birth to 5886 infants conceived following ART treatment were compared to a reference population of 245249 mothers who gave birth to 248539 spontaneously conceived infants delivered in Australian hospitals in 2003. Three national data collections (Australian and New Zealand Assisted Reproductive Database (ANZARD), National Perinatal Data Collection, National Hospital Morbidity Database) were used to construct a costing model of maternal and infant birth-admissions. Costs were calculated using Australian Refined Diagnosis Related Groups.

Results: ART infants were 4.4 times more likely to be low birth weight compared to non-ART infants, translating into significantly higher birth-admission costs. ART singletons were also more likely to be low birth weight compared to non-ART singletons, translating into higher maternal age-adjusted birth-admission costs. The combined infant and maternal costs of ART twin and higher order multiples were 3 and 11 times higher than for ART singletons. Findings were not sensitive to changes in casemix.

Conclusion: The healthcare cost of ART singletons were higher than non-ART singletons, but substantially less than ART multiple births, adding to the overwhelming clinical evidence in support of single embryo transfer.

Reference

1. Wang Y, Dean J, Grayson N, Sullivan EA (2006). Assisted reproduction technology in Australia and New Zealand 2004. Assisted reproduction technology series no 10 Cat no PER39. Sydney: AIHW National Perinatal Statistics Unit.

Infertility Demographics in Australia and New Zealand

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The incidence of infertility, 1 in 6 couples, appears to have not altered for the past 3 generations. First and subsequent pregnancies are equally affected. However, societal changes over the past three decades have resulted in couples having a significantly reduced time in which to conceive. The average age at marriage in Australia for women is 29.7 years and 32 years for men (Australian Bureau of Statistics, 2006 Census data). This means a couple has a very limited time to conceive compared to 30 years ago when the average age at marriage was 22 to 23 years.

The trend for an increasing number of adults in their 20's to still be living at home (68% in Australia – 75% of men and 51% of women) is a significant factor in the later age of adults partnering and then procreating.

The individual impact of male and female increasing age on fertility is not well understood by couples or healthcare providers.

Common conditions that affect both fertility and also long term health – polycystic ovaries, endometriosis and Klinefelter's syndrome – are significantly under diagnosed.

Lifestyle factors that affect fertility such as smoking and alcohol intake have reduced little despite increasing knowledge

of their negative impact. Increased weight, another adverse fertility factor, has increased rapidly, so that 35 to 50% of women and 50 to 70% of men in the reproductive age group are now overweight or obese.

A fertility preservation education programme for adolescents, adults and their primary healthcare providers is required to reverse these factors.

Double Trouble or Fun With One?

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Aim: To compare the fresh and frozen pregnancy rate from a single stimulated cycle after one or two embryo transfer.

Method: 771 patients aged under 40 (excluding embryo biopsy, donor gametes and hyperstimulation) having a day 3 transfer, were retrospectively analysed using Chi-Squared.

The elective Single Embryo Transfer (eSET, n = 209) and elective Double Embryo Transfer (eDET, n = 562) groups were defined as one or two embryos transferred respectively with at least one day 3 embryo frozen.

For a comparative analysis of eSET vs. eDET, cycles containing at least 3 utilizable embryos were examined. That is, an eSET with ≥ 2 embryos frozen (n = 186) compared to eDET with ≥ 1 embryo frozen (n = 562).

Results: Analysis of fresh pregnancy only, eDET showed a pregnancy rate of 42.0% compared to eSET of 33% (p = 0.001). When at least one FET cycle was included the pregnancy rates equalised (eSET = 57.4% cf. eDET = 55%). The latter had a 28.5% likelihood of multiple pregnancy. The eSET group contained 3 sets of monozygotic twins (1.4% twinning).

Analysis of the subset containing 3 utilisable embryos yielded equal cumulative pregnancy rates (eSET = 60% cf. eDET = 55.5%).

Conclusion: In order to circumvent the problem of multiple pregnancies in IVF patients, this study has shown that patients can choose to have one embryo transferred at a time without compromising cumulative pregnancy rate, compared to a double-embryo transfer. For this to be viable, a patient needs one excess utilizable embryo.

Impact of Blastomere Lysis on Sibling Blastomere Cleavage in Frozen/Thawed Embryos

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Aim: To determine whether lysed blastomeres in frozen/thawed embryos influence subsequent cell division in adjacent surviving blastomeres.

Methods: Retrospective analysis of the frequency of individual blastomere cleavage in embryos frozen at the 4 cell stage

on day 2, thawed and cultured overnight prior to transfer. Statistical analysis was by χ^2 .

Results: From a total of 1178 thawed embryos, 691 (59%) survived with all 4 blastomeres intact, 169 (14%) contained a single lysed blastomere and 97 (8%) contained 2 lysed blastomeres. The remaining 221 embryos (19%) with less than 50% surviving blastomeres were considered unsuitable for clinical use and were discarded prior to overnight culture. In the fully intact thawed embryos 59.7% (1650/2764) of the blastomeres underwent subsequent overnight cleavage. The proportion of individual surviving blastomeres which underwent subsequent overnight cleavage was significantly ($p < 0.001$) reduced when thawed embryos contained a single lysed blastomere (231/507; 45.6%) and when 2 lysed blastomeres were present (75/194; 38.7%).

Conclusion: The presence of lysed blastomeres in thawed embryos appears to inhibit subsequent cleavage in adjacent surviving blastomeres. The extent to which this inhibition, rather than the reduction in cellular content per se, is responsible for reduced implantation potential warrants further investigation.

Oocyte Donation: Results of a Known and Unknown Donor Oocyte Programme over Five Years

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Aim: To present outcome data of a successful oocyte donation programme, and some comments by donors.

Method: Review of outcome data of 193 known and 121 anonymous oocyte donors and recipients in the years 2002 to 2006 at a major fertility clinic.

Results: 193 known donors undertook 216 oocyte retrievals. The average age of known donors was 32.9 years, ranging from 19–42 years. 121 anonymous donors undertook 179 oocyte retrievals. The average age of anonymous donors was 32.2 years, ranging from 22 to 38 years. A combined 314 fresh embryo transfers led to 125 pregnancies (39.8%), with 113 of 233 recipients gaining a pregnancy (48.5%), resulting in 59 live births and 13 ongoing pregnancies. A combined 372 frozen embryo transfers (FET) led to 101 pregnancies (27.2%) and a take home baby rate of 19%. Of the 179 FET recipients, 87 became pregnant (48.6%), with 65 patients having a live birth, and six having an ongoing pregnancy. Comments about reasons for donating are included. All anonymous donors were fully consenting to future identification and completed an extensive personal profile to be given to recipients.

Conclusion: A known and anonymous donor oocyte programme presents long-term infertile clients with a positive opportunity of achieving their own children from donated oocytes. Oocyte donation did not appear to have any of the stigmas attached to sperm donation, with women donating even with the intrusive nature of oocyte retrieval.

Embryo Donation: Results of an Unknown Embryo Donation Programme over Five Years

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Aim: To present outcome data of a successful embryo donation programme, and some comments by donating couples.

Method: Review of outcome data of 112 anonymous embryo donations and 83 recipients in the years 2002 to 2006 at a major fertility clinic.

Results: 112 embryo donations resulted in 153 thaws, and 136 frozen embryo transfers. 36 pregnancies were achieved, with a pregnancy rate of 26.7% per transfer. 81 patients received one or more transfers, 33 gaining one or more pregnancies (40.7%). 36 pregnancies resulted in 22 live ongoing babies, one neonatal death, one stillbirth, 12 miscarriages, and four ongoing pregnancies. Donating women were aged between 25 to 41 at time of embryo formation, with a mean of 34.7 years. Couples donated from 7 months to 4 years after their last birth, with a mean of 2.3 years. During this period 642 clients ceased embryo storage, with 112 donating, a donation rate of 17%.

Donating couple comments indicated the reasons for donating varied from their family being complete to an opportunity to help someone become parents. Donors had thought well and truly about donating over a long period. Donors recognized their genetic connectiveness but were happy and comfortable about anonymous donation. All donors fully agreed to future identification and completed extensive personal profiles to be given to the recipients.

Conclusion: 17% of couples disposing of excess embryos donated to other couples. Of 81 recipients, twenty achieved a take home baby, with four ongoing pregnancies. Anonymous embryo donation is positively viewed and utilized by both donors and recipients.

Assessment of Ovarian Perifollicular Blood Flow during IVF Treatment

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Aim: To examine ovarian perifollicular blood flow (PFBF) during the follicular phase in women undergoing IVF.

Method: Prospective longitudinal observational study involving serial assessment of ovarian PFBF in all follicles >4mm using Power Doppler ultrasound during the follicular phase of IVF treatment. Ovarian PFBF was subjectively assessed using a grading system (Grade 0: 0% of follicular circumference, Grade 1: 1–25%, Grade 2: 26–50%, Grade 3: 51–75%, Grade 4: >75%). Ovarian PFBF grades were categorized as presence of PFBF (1–2–3–4) and high grade PFBF (3–4). Follicles were categorized as small (5–10mm), medium (11–14mm), and large (≥ 15 mm).

Results: Thirty-four women undergoing a single IVF treatment cycle were enrolled and 1050 ovarian follicles were used

for data analysis. Throughout the follicular phase, 81% of follicles had grade 0–1 vascularity. High grade PFBF was observed during the entire follicular phase in 5% of total follicles. There was a greater proportion of follicles with high grade PFBF with increasing follicular development from small (2.2%) to medium (7.3%) to large (9.2%) follicles. A highly significant positive correlation was seen between the size of ovarian follicles and both the presence of ovarian PFBF (Pearson's $r = 0.796$; $p < 0.0001$) and high grade ovarian PFBF (Pearson's $r = 0.220$; $p = 0.004$).

Conclusion: This study demonstrates an improvement in ovarian PFBF with increasing follicular development and size over the course of the follicular phase in infertile women undergoing ovarian stimulation during IVF treatment. High grade PFBF was observed in less than 10% of follicles, regardless of size.

A Prospective, Randomised, Double-Blind, Placebo-Controlled Trial Of Multimodal Intra-Operative Analgesia For Laparoscopic Excision Of Endometriosis

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Aim: To assess the efficacy of multimodal intraoperative analgesia in reducing post-operative pain and/or opioid requirements in women undergoing laparoscopic excision of endometriosis.

Method: Random assignment of 66 women undergoing laparoscopic excision of endometriosis to receive intraoperative multimodal analgesia (30 patients) or placebo (36 patients). Analgesia consisted of Diclofenac sodium 100mg suppository per rectum and 0.75% Ropivacaine to portal sites, sub-peritoneally under excision sites and topically to each sub-diaphragmatic area. Post-operative in-hospital analgesia was standardized for all patients and included IV morphine delivered by patient controlled analgesia (PCA) in the ward. The primary outcome measures were (1) postoperative opioid analgesic requirements and (2) postoperative pain intensity measured by Visual Analogue Scale (VAS) and Verbal Descriptor Scale (VDS).

Results: There was no difference in baseline variables between the two groups. The analgesic group used significantly less morphine in recovery (0.0V 8.0mg; $p = 0.016$), PCA morphine in the ward (9.0V 21.5mg; $p = 0.05$), and total hospital opioid (recovery morphine, PCA morphine and ward breakthrough opioid) (19.0V 34.5mg; $p = 0.017$) compared to the placebo group. Results are presented as medians. There was no difference in post-operative pain intensity between the two groups.

Conclusion: The use of multimodal intra-operative analgesia at laparoscopic excision of endometriosis reduces post-operative opioid consumption.

Birth Outcomes after Vitrification and Slow Freezing of Supernumerary Blastocysts

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Aim: A successful cryopreservation protocol is essential to reach the full potential for any single IVF stimulation cycle while still reducing the risks associated with multiple gestations. This study compares live birth outcomes from embryos that were slow frozen or vitrified.

Method: Since January 2006, all supernumerary blastocysts were vitrified using the Sydney IVF Vitrification Media suite (16% DMSO, 16% Ethylene Glycol and 0.68M Trehalose) and the CVM Kit (Cryologic, Australia). Embryos were thawed at 37°C in decreasing concentrations of Trehalose. Prior to this, blastocysts were frozen using a controlled rate freezer (Planer, UK). Embryos were thawed and passaged through Thawing Media. All embryos were laser hatched immediately after thawing (Zilos, Hamilton Thorne). Birth outcomes were recorded for embryos thawed and transferred between March and June 2006. During this period, 101 vitrified embryos were transferred in 86 cryo-cycles and 208 slow frozen embryos were transferred in 166 cryo-cycles. The data were analysed using a comparison of proportions test.

Results: Compared to slow frozen embryos, vitrified embryos resulted in higher biochemical, fetal heart and live birth rates (48% (41/86) cf 32% (53/166) for bHCG +ve ($P = 0.02$); 35% (34/86) cf 19% (36/166) for fetal heart pregnancies ($P = 0.01$) and 34% (29/86) cf 18% (30/166) for live births ($P = 0.009$)). The average number of embryos transferred was similar for vitrified embryos (1.2) and slow frozen embryos (1.3).

Conclusion: Vitrification is a successful cryopreservation method that maintains good embryo viability. These results should help to reduce patient anxiety about IVF success while encouraging clinicians to use elective single embryo transfer (eSET) whenever possible.

In-Vitro Maturation (IVM) and Development of Nonhuman Primate (NHP) Oocytes: Oocyte Glutathione (GSH) and Culture Environment

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Aim: To pharmacologically enhance oocyte GSH levels and improve IVM and post-fertilization development of immature NHP oocytes collected following controlled ovarian stimulation (COS).

Method: IVM of MI oocytes collected after COS of Macaca fascicularis ($n = 20$, WaNPRC study approval #3387-01). Four IVM media (A) HTF (B) mCMRL (C) mCMRL + 3mM GSH ethyl ester (OET3) and (D) mCMRL + 5mM GSH ethyl ester (OET5) were examined for their effects on MI-MII maturation, fertilization and blastocyst development of oocytes exhibiting early (4–6 hours) and late (18–20 hours) polar body

extrusion following collection. Data was analysed using ANOVA, Chi-square or Fisher Exact (significance, $p < 0.05$).

Results: GSH levels in early oocytes cultured in HTF (1.82 ± 0.16 pmol/oocyte, $n = 16$), mCMRL (3.51 ± 0.35 pmol/oocyte, $n = 59$) and OET5 (5.04 ± 0.64 pmol/oocyte, $n = 68$) were significantly different. Maturation rates of early oocytes were not different across treatments (20–32%). Early oocytes cultured in OET5 had significantly higher 2PN (89.7%) and lower 1PN (7.1%) rates compared to HTF culture (50%, 21.4%, respectively). Total cell counts in OET5 blastocysts (204.8 ± 22.4 , $n = 8$) were significantly higher compared to mCMRL (140.1 ± 7.3 , $n = 5$) blastocysts. OET produced significant concentration dependent improvements in maturation (88–89%), 2PN (72–84%) and 1PN (0–8%) rates of late MI-MII oocytes as compared to mCMRL alone (73%, 43% and 22%, respectively).

Conclusion: Supplementation of IVM media with OET enhances oocyte GSH content and improves developmental outcomes of MI-MII oocytes in a clinically relevant nonhuman primate model for human oocyte maturation.

Pregnancy in a Non Communicating Rudimentary Horn

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Aim: To review a 32-year-old woman who had a pregnancy in a rudimentary uterine horn.

Background: A patient presented with post pill amenorrhoea at 28 years of age. An HSG was performed as part of routine investigations, and this displayed failure to fill the right tube. She proceeded to have a HyCoSy scan which showed the appearance of a lateral right mass, with no fill or spill on this side. She had 6 months treatment with clomiphene citrate, and ovulated twice during this treatment, but did not achieve a pregnancy. She then proceeded to have a successful ovulation induction with gonadotrophins. She delivered a term infant with spontaneous onset of labour, and forceps delivery.

For her next pregnancy she conceived without assistance. An early ultrasound was suspicious for ectopic pregnancy in the right adnexa, and there was a corpus luteum present on the right ovary. After review of the scan images, and comparison to the HyCoSy, an MRI was performed. This showed a pregnancy implanted in a non-communicating rudimentary horn.

Methods and Results: The gestational sac in the rudimentary horn was injected with methotrexate with a spinal needle under ultrasound guidance, and the HCG levels returned to normal. The rudimentary horn was subsequently excised laparoscopically.

Conclusion: Without communication between the 2 uterine horns, the likelihood of sperm transport through the peritoneal cavity is a likely explanation for pregnancy in the rudimentary horn, especially in the presence of the corpus luteum on the right ovary.

The absence of a tubal fill and spill with a lateral mass should alert the clinician to the possibility of a rudimentary

horn, and this should be excised prior to attempting conception or fertility treatment.

Abstract withdrawn

Testing Ovarian Reserve: Women's Experience of Receiving Poor Results

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Aim: The introduction of Ovarian Reserve testing to the general population in Adelaide in 2004 was accompanied by concern about the effect to women's wellbeing of receiving results that were poor. An underpinning assumption of ovarian reserve testing was that becoming aware of her ovarian reserve would then allow a woman to make more informed reproductive decisions. This pilot study aimed to explore the experience of women who had undergone an Egg Timer test since it was publicly launched.

Method: Twelve women and one man aged 22–43, were interviewed, and the interviews recorded and transcribed verbatim. Participants were asked to describe how they were referred for the test, their experience of having the procedure and receiving results, and how the knowledge gained affected their lives and reproductive decisions. The narrative data was coded and analysed thematically.

Results: Several of the women had a prior concern about their fertility. All participants expressed a high degree of confidence in the capacity of the test to predict their reproductive future. Women whose ovarian reserve was poor described a profound impact to their reproductive and career plan, their primary relationship, family and to their sense of self. The receipt of poor results precipitated a 'crisis of readiness'. Although all women

were referred by a GP, this doctor remained peripheral to their distress.

Conclusion: The information that a woman has poor ovarian reserve should be delivered with care, an offer of counselling referral and psychosocial follow-up. General Practitioner referral does not guarantee this will occur.

Factors and Perceptions That Influence Women's Decisions to Have a Single Embryo Transferred

(Poster Presentation)

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¹Research Centre for Reproductive Health and Repromed, ²Data management and analysis, Department of Public Health, Adelaide University, Adelaide, Australia

Aim: Multiple pregnancies following In Vitro Fertilisation and Intracytoplasmic Sperm Injection (IVF/ICSI) are generally considered to be iatrogenic events since a multiple gestation is associated with peri-natal mortality and infant morbidity. In our clinic there has been a strong movement towards reducing the risk of multiple pregnancy, but this movement is reliant on women's informed choice. The aim of this study was to identify factors that inhibit or promote the adoption of SET in a cohort of women during an IVF/ICSI treatment.

Method: One hundred & sixty three IVF/ICSI women patients were interviewed by telephone using a structured questionnaire.

Results: Eighty-seven women choosing SET and 63 women choosing DET were compared using logistic regression analysis. Confidence in the chance for pregnancy with SET, younger age and first treatment emerged as important variables predictive of a decision for SET. In the choice of SET personal preferences for a healthy and singleton pregnancy was predictive, but perceptions of the incidence or risks of multiple gestation associated with multiple embryo transfer were not. Factors such as a sense of time urgency and past experience of treatment were significant factors that diminished the likelihood of a choice for SET. The Clinic Doctor was an important influencing factor.

Conclusion: Improved pregnancy rates in SET coupled with an official clinic policy to promote SET in younger, first cycle patients influenced many women to choose SET. However repeated treatment, advancing age and urgency to become pregnant are factors that moderate a woman's choice for SET.

Family Formation in the 21st Century

Deb DEMPSEY

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The nuclear family model, characterised by marriage in young adulthood, high fertility and clear gender role differentiation, reached its high point in the Post-World War Two era. However, in the past 30 or so years, newer family forms including single parent, and lesbian and gay parented families with children

have proliferated. Changing social expectations of appropriate gender roles and sexual identities, economic factors, government policies and technological advances such as ART all influence the perceived possibilities when it comes to bringing children into the world.

Media debates about unconventional family formation tend to emphasise two main stories about the family. For some members of the community, the prospect of creating single parent or lesbian and gay families through use of donor insemination, surrogacy and IVF is evidence of family breakdown rather than inevitable change. Others hold what could be called a more optimistic view. From this perspective, while there may be some new social and legal dilemmas to come to grips with as unconventional family forms proliferate, overall, they reflect new opportunities for living in caring and respectful relationships. In other words, family is finding new expressions rather than breaking down.

In this paper, I sketch out the concerns about family change evident in media debates about single and lesbian parent families, arguing these indicate a range of complex preoccupations with contemporary Western families and parenthood. I then consider how sociologists conceptualise changing families as giving rise to 'new imaginaries of responsibility' about relationships and parenthood beyond the nuclear family, drawing on the social research evidence that informs this more optimistic viewpoint.

Establishment of a Nurse-Based System for Conducting Randomised Clinical Trials in a Large IVF Network

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Aim: Properly conducted randomised controlled clinical trials (RCTs) are an essential component of high quality innovation in infertility practice. At IVFAustralia, we set out to design a practical system, for conducting RCTs, that would be fully compliant with CONSORT (1).

Method: A short multi-disciplinary Case Record Form (CRF) was developed to direct a common system for recruitment, randomisation and data collection, for application to multiple RCTs. The clinician ascertains eligibility and makes the initial approach, at medical consultation. The trial nurse follows the patient up by telephone to answer questions and check eligibility. Informed consent is obtained at presentation for treatment. Randomisation is performed by any trained staff member, at the point defined by the study, using a specifically designed website for proper randomisation including adequate concealment of allocation. At conclusion of the treatment cycle, the trial nurse enters any required data, that is not stored in the clinic database, into the CRF and records all protocol violations and adverse events on the trial website. The website allows investigators to monitor progress of the study while maintaining concealment of primary study endpoints.

On conclusion of the study, defined endpoint data are downloaded from the organisation's routine database for analysis.

Results: This system is currently in use for one RCT with two others close to initiation. At time of submission, 70 patients had been studied in the first IVFAustralia RCT.

Conclusion: An effective system can facilitate randomised controlled trials in a large IVF network.

Reference

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Building Strong Donor Families

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Dr. Ehrensaft addresses the challenges to family building when baby is conceived with the help of a “birth other,” a term coined for the person who donates gametes or allows use of her uterus to help someone else have a child. Starting with the premise that “there is no baby without the people who make the baby and the people who raise the baby,” the psyches of all participants are taken into account in looking at the dialectic between hope and fear, acceptance of a baby made from science rather than sex, and realization that the gametes or womb are connected to a human being who must be factored into family life. Laying claim to the baby is discussed in the context of genetic asymmetry between parents and fears of birth other transformed in fantasy into conniving kidnapper or sexual intruder. Psychological defense mechanisms, including “immaculate deception,” are analyzed as coping strategies for dealing with the birth other as both an “extra” piece and “missing” piece of the family, along with societal “reproductive technophobia” as it affects parents’ confidence in providing a safe, supportive environment for their children. The “family reverie,” in which parents and children talk freely together about the birth other, along with its psychological companion, the “family romance,” in which children are allowed their own private thoughts about the birth other, are presented as strategies to strengthen attachments, build intimacy, and fortify the children’s identity as a “birth other” child.

Randomised Control Trial of Ovarian Stimulation with Intrauterine Insemination Versus In Vitro Fertilisation Using Identical Stimulation Protocols

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Aim: To compare the results of intrauterine insemination (IUI) with controlled ovarian hyperstimulation (COH) with those undergoing IVF using the same stimulation protocol in couples with poorly explained infertility or mild male factor infertility on the primary outcome of pregnancy rate with documented fetal heart using a randomised control trial study design.

Method: Prospective randomised control trial in which one hundred and two eligible infertile patients were recruited over

a period of 3 years. Only patients who produced 2–3 follicles on ultrasound prior to HCG administration and following minimal stimulation protocol using 112.5IU of FSH were included in the study. Forty one patients were randomised to either IUI or IVF by opening a sealed envelope with a ratio of 3:1 (31 patients in IUI arm of the study and 10 patients to IVF arm of the study. Pregnancy rate was documented and fetal heart was compared using a Chi-squared test with $p < 0.05$ considered to be significantly different.

Results: The main outcome measure is the pregnancy rate with documented fetal heart. Pregnancy rates for both the IUI and IVF groups were 9.6% and 40% respectively with a statistically significant difference between two groups, $p = 0.03$.

Conclusions: IVF should be offered as a primary treatment of poorly explained infertility or mild male factor.

Knowledge and Attitude Towards Egg Donation Among Egg Recipient Couples in Iran

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Aim: Today, assisted reproductive techniques are practiced in almost all around the world and in many places donation program is routine. In the Middle East countries and Islamic contexts, donation and surrogacy is forbidden except Iran. So, as a unique Islamic country which egg, sperm and embryo donation are allowed, it is very important to know about knowledge and attitude of the people involved in the program. This study has been designed to check the knowledge and attitude of egg recipient women and their husbands towards egg donation.

Method: The study was a cross sectional study in 2005 on 29 egg recipient women and their partners using a well controlled questionnaire. The excluding criteria was unwillingness of the couples and the data was analyzed using SPSS.

Results: The response rate was 100%. 96% of the recipient women were happy about having a child by egg donation but this percent was 89.1% among their husbands. A large portion of the couples were in agreement with donor compensation and equality of these children’s rights with the others. 79.9% of the women wanted to know the donors but this value was only 34.4% among the men and the difference was significant ($p: 0.002$). Majority of the couples were against: getting eggs from the recipient’s relatives, to come and go with the donor, informing their father- or mother-in-law, informing the offspring about the donation and introducing the donor and registration of the names in a national center. 41% of the couples accepted the intimacy of the offspring with the donors and 58% of men and 48% of the women were against any legal rights between the donor and their offspring.

Conclusion: It seems that anonymity of the donor is well accepted in Iran, although is not observed. Legal and religious knowledge is lacking that needs an educational program for these couples. Also, informing the offspring or close relatives is not acceptable by majority of the couples.

Long Term Follow Up of Women with Laparoscopic Ovarian Diathermy for Women with Clomiphene Resistant Polycystic Ovarian Syndrome

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Aims: The aim of this report was to establish the long term benefits and harms of LOD in the group of women who took part in a RCT 6–10 years earlier outcomes.

Methods: Women who took part in the fertility PLUS randomised controlled trial comparing laparoscopic ovarian diathermy and gonadotrophins were contacted 6–10 years following the end of the study. A postal questionnaire of clinical symptoms and pregnancy outcomes was collected and FSH levels measured.

Results: Of the 33 women who completed the postal questionnaire, twenty-nine had undergone LOD. The mean FSH level of these women is 5.7IU/L (SD 3.7) ranging from <0.1 to 18.4IU/L. The number of periods per year increased from 3.5 at study entry to 7.8 in the follow up period in women not on the oral contraceptive. At the follow up, 15 of 29 women undergoing LOD (52%) reported symptoms of hirsutism and 14.0% reported acne. After undergoing laparoscopic ovarian diathermy 79.0% (23/29) of the women delivered live infants and over the follow up period 35 babies were delivered. Further fertility treatment was needed by 14 of 29 women who underwent LOD.

Conclusions: Six to ten years after LOD, ongoing fertility and regular menstrual cycles were present in more than half of the women who underwent LOD.

A Comparative Review of Pregnancy Rates in Women with Group 1 WHO and Group 2 WHO Ovulatory Infertility

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Introduction: Ovulation induction (OI) using gonadotrophins has been an effective way of treating couples with infertility associated with ovulation disorders since its introduction in the 1970's. The Ovulation Induction (OI) clinic in our unit was set up in 1978 and has managed over 4000 cycles since its inception. A retrospective analysis was performed looking at the differing types of ovulation disorders and their pregnancy rates using the various preparations of gonadotrophins made available since that time.

Materials and Methods: All patients attending the OI clinics were entered into a database (FoxPro for Windows), including patient demographics, medical history, and types of treatment used to date and a detailed endocrine profile in response to each treatment cycle. A retrospective analysis was performed.

Patients were classified according to the WHO classification of ovulation disorders. (Group 1 = anovulation lasting for >6 months and Group 2 = irregular ovulation)

Results: 1560 patients attended the OI clinic between 1981 and 2006, undergoing 4197 cycles of OI using gonadotrophins. There were 850 treatment cycles of OI for patients with anovulation >6 months and 1231 cycles of OI for patients with irregular ovulation. The majority of patients fell into Group 1 and 2 WHO categories. Gonadotrophin preparations included human menopausal gonadotrophin (hMG), purified urinary FSH, highly Purified urinary FSH and recombinant FSH. The patient mean age for Group 1 was 30 years and Group 2 = 32 years (range 19–49). The average amount of FSH required was 700.8 units with a large observed difference required in both groups (Group 1 = 1412.9 units per cycle; Group 2 = 659.5 units/cycle). The pregnancy rate for patients with anovulation >6 months was 22.1% and 15.9% for patients with irregular ovulation. The highest pregnancy rate was observed in the recombinant FSH group for both WHO groups 1 and 2. The observed difference between each gonadotrophin preparation for pregnancy rates reached statistical significance for WHO group 2.

Conclusions: The method by which the use and monitoring of gonadotrophins in OI programmes has evolved has continued to make its use very favourable and efficient for couples with ovulatory infertility. The transformation to recombinant FSH alone has improved pregnancy rates and appears to have reduced the incidence of miscarriage in all patient groups. Allowing for subcutaneous administration of recombinant FSH and shorter time frame in which reproductive hormones can now be assayed; patients find treatment less invasive and OI protocols easier to follow.

First Prospective Controlled Trial of a Novel Membrane-Based Electrophoretic Method of Isolating Spermatozoa for IVF

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Aim: A novel system of membrane-based electrophoretic filtration that isolates spermatozoa with significantly less DNA damage has recently been developed (1). However, it is not known whether electrophoretic preparation of spermatozoa impairs their ability to achieve fertilisation or yield good quality embryos in IVF.

Method: So far, 12 patients have been recruited onto this trial. Variations in both oocyte and semen quality were controlled for as follows: The semen sample was split evenly for preparation by either density gradient centrifugation (DGC) or by electrophoresis (Gradiflow[®] CS-10, NuSep Ltd). Both sperm preparations were washed and diluted to yield the same concentration of motile spermatozoa. Oocytes (n = 183) retrieved from each patient were evenly and randomly allocated for insemination using sperm prepared by each method. Fertilisa-

tion rates, cleavage rates, and embryo quality post-insemination (pi), were compared using two-sided ANOVA, a p value <0.05 being considered statistically significant.

Results: No significant difference between spermatozoa prepared by DGC and electrophoresis was observed with respect to subsequent rates of fertilisation (82% vs 74% 16hpi; $p = 0.221$), cleavage (92% vs 100% 40hpi; $p = 0.128$), and top grade day-2 embryos (36% vs 45% 40hpi; $p = 0.500$), respectively.

Conclusion: This is the first, prospective, split-cohort, split-sample, controlled trial for the application of this novel technique of sperm preparation in IVF. These preliminary results suggest that membrane-based electrophoresis is a reliable and efficient means of preparing spermatozoa for IVF.

Acknowledgement

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Reference

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Early and Late Events in Embryo Development are the Best Predictors of Outcome

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Aim: To analyse the efficacy of a number of developmental parameters in selecting the most viable blastocyst with single embryo transfer (SET).

Materials and Methods: All day 5 SETs, with or without subsequent freezing (eSET and non-eSET, respectively) were retrospectively analysed. PGD and donor egg cycles were excluded.

Normally fertilised zygotes were analysed approximately 24hpi for early cleavage (2PN, 1C syngamy, 2cell). Embryos were then assessed daily with a single embryo transferred on day 5.

For the purpose of this study blastocysts were categorised into two groups; early blastocysts (EB) a small blastocoel with no apparent embryo expansion, and advanced blastocysts (AB) a large blastocoel and visible embryo expansion.

Results: A total of 284 SETs were analysed. The overall pregnancy rate was 41.5%. No significant age difference was found (≤ 39 yo 41%; ≥ 40 yo 43%). For eSET, the clinical pregnancy rate was 48% compared with 24% for non-eSET ($p = 0.001$).

With all SET, transferring an EB on day 5 resulted in significantly fewer pregnancies compared with AB (31.4% and 51%, respectively $p = 0.001$).

Zygotes in syngamy 24hpi which resulted in EB transfer produced significantly fewer pregnancies compared with embryos that cleaved 24hpi (18% and 50%, respectively $p = 0.002$).

There was no correlation with pregnancy outcome and zygote status 24hpi when an AB was transferred.

Embryo development on day 2, 3 and 4 and degree of fragmentation on day 3 produced no significant correlations with pregnancy outcome.

Conclusion: Early cleavage, together with the degree of blastulation appear to be the most reliable tools in selecting the most viable blastocyst for transfer. Cell stage on day 2, 3 and 4, and degree of fragmentation on day 3 are poor indicators of blastocyst development and subsequent pregnancy potential.

Preservation of Professionals – Prevention of Burnout

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Burnout is a psychological syndrome that develops after prolonged exposure to chronic stressors in the workplace. The prevalence of burnout across various helping professions averages between 6 & 11%, with rates over 20% reported in some contexts. The costs of burnout can be high both for the individual and the workplace. For the individual there can be longer term emotional difficulties such as anxiety and depression, increased physical illness, increased drug and/or alcohol use, work avoidance and overall questioning of career suitability. For the organisation, workplace burnout can result in increased staff dissatisfaction, unexpected leave, absenteeism, high staff turnover rates and ultimately higher training and employment costs. The 'preservation of professionals' through burnout prevention is a goal which benefits all.

In this presentation I will outline the burnout syndrome in more detail as well as the risk factors for burnout development, both from an individual and organisational perspective. The implications of these risk factors for burnout prevention strategies will also be discussed, again in terms of both individual and organisational interventions. Finally, the findings from some recent research into the levels and predictors of burnout in a group of Australian allied health professionals will be presented.

Infertility 'No Issue' for Maori

Marewa GLOVER

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Rather than groping in the dark for new conceptual tools to make sense of the perceived social challenges introduced by assisted human reproduction (AHR), people can draw on existing cultural resources. Using material drawn from a study of Māori (the indigenous people of New Zealand) attitudes to AHR, Dr Marewa Glover explores the frameworks used by Māori to evaluate the place of AHR in their lives, families and communities.

A Māori research methodology allowed for the research to be conducted in accordance with Māori cultural protocols. Using a naturalistic qualitative design and a general inductive analytical method further enabled Māori perspectives to emerge. Sixty-five Māori participants from diverse backgrounds (including key informants, youth, elders, health workers, takatāpui [non-heterosexuals], men, and Māori who had experienced infertility) were interviewed either individually or in focus groups.

The findings of the study indicate that the participants did not experience making use of their existing cultural resources to explain the novel implications of AHR as a “foreign” process. Dr Glover will explain how Māori think about AHR through the lens of their main family and tribal groupings, extant cultural practices (such as whāngai/alike to adoption); and essential cultural resources (whakapapa/genealogy). Participants considered infertility an introduced concept which will require reassessment of existing cultural discourses of reproduction and fertility.

The research showed how cultural models and resources can be discovered in order to inform the inclusive development of more appropriate policy regarding the provision of reproductive services to indigenous people.

Comparison of Slow Freezing and Vitrification of Human Oocytes

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Aim: The aim of the study was to compare current slow freezing methods with vitrification using in-vitro matured human oocytes.

Methods: Human germinal vesicle stage oocytes, collected from stimulated cycles, were cultured in Quinns Fertilization Medium to the Metaphase II stage. Oocytes were subsequently frozen using one of 4 cryopreservation methods and thawed/warmed using the appropriate method:

- A. Slow freezing in 1.5M PROH and 0.2M sucrose (dehydration and rehydration at 37°C)
- B. Slow freezing in 1.5M PROH and 0.3M sucrose (dehydration and rehydration at 22°C)
- C. Vitrification (1) using Kitazato vitrification kit
- D. Vitrification (2) using 2.7M Ethylene Glycol + 2.1M DMSO + 0.5M sucrose

Survival was assessed after 2 and 24 hours, and results analysed using χ^2 .

Results:

Method	A	B	C	D
Number of oocytes thawed/warmed	19	13	17	16
Number of oocytes recovered	19	13	16	15
Number of oocytes surviving (%)	16 (84)	11 (85)	15 (94)	14 (93)

Conclusion: Both slow freezing protocols resulted in similar survival. Similarly, there was no difference ($p > 0.5$) in survival between the two vitrification protocols. Although vitrification appeared to result in higher survival than slow freezing, there was no significant difference ($p > 0.5$) in overall post thaw oocyte recovery. This preliminary study shows no significant difference in survival between the four cryopreservation methods assessed.

New News on Birth Defects and Assisted Reproductive Technologies

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¹Murdoch Childrens Research Institute, Parkville, ²Monash IVF, Richmond, ³Melbourne IVF, Royal Women’s Hospital, ⁴Monash University Department of Obstetrics and Gynaecology, Clayton, Victoria, Australia

Aim: To determine the risk of birth defects associated with different ART procedures and type of embryo transfer (fresh or frozen) in singletons.

Methods: This retrospective record linkage cohort study covered the years 1991–2004. Exposure groups were women having IVF (n = 4190), or ICSI (4, 289), with fresh or frozen embryo transfer. The controls were randomly selected women having non-ART births from the Victorian Perinatal Data Collection Unit, matched on maternal age and year of birth (25, 437). Logistic regression models, estimated using Generalised Estimating Equations to allow for correlation within sibships, were fitted to estimate odds ratios for birth defects, adjusting for potential confounders (maternal age, year of birth, number of previous abortions, sex of baby).

Results: Compared to non-ART controls, the odds of a birth defect was increased for ICSI with either frozen (OR 1.65, 95% CI 1.33, 2.04) or fresh (OR 1.46, 95% CI 1.22, 1.73) embryo transfer, and for IVF fresh (OR 1.44, 95% CI 1.21, 1.73), but not frozen (OR 1.03, 95% CI 0.82, 1.29) embryo transfer. Specific birth defects demonstrated associations, e.g. chromosomal and genitourinary with ICSI frozen embryo transfer.

Conclusions: A new finding amongst women having ART was that, compared to non-ART women, there was no increased likelihood of birth defects when IVF was followed by frozen embryo transfer procedure. The highest risk for birth defects was found amongst those having an ICSI procedure followed by frozen embryo transfer, while both ICSI and IVF followed by fresh embryo transfer procedure were associated with an almost identical increased risk.

Adverse Perinatal Outcomes of Subfertile Women Identified on a Birth Register in Victoria

(Poster Presentation)

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Aim: To investigate whether subfertility is associated with adverse perinatal outcomes.

Methods: Using probabilistic record linkage, couples who registered at two Victorian infertility clinics between 1991 and 2000 but had unsuccessful or no ART treatment

(IVF/ICSI/GIFT), were matched to birth records at the Victorian Perinatal Data Collection Unit (PDCU). A control group of births to 'fertile' women was randomly selected from the PDCU. Multivariate logistic regression adjusted for potential confounders (e.g. maternal age and gender).

Results: After excluding subsequent births and babies conceived with ART at other clinics, we identified a first born singleton to 18% of all registrations who had no or unsuccessful ART. Compared to fertile controls, being subfertile and having no ART treatment was associated with having a baby with a birth defect (AdjOR = 1.5, 95% CI 1.1, 2.1). 72% of this group conceived within 6 months of registration. Having unsuccessful treatment was associated with preterm birth (AdjOR = 1.6, 95% CI 1.2, 2.0) and 80% of these women conceived after 6 months. Overall, subfertile couples who took 6 months or more to conceive after registration had an increased likelihood of delivering preterm (AdjOR = 2.3, 95% CI 1.4, 3.9), but no increased risk of birth defects.

Conclusions: Subfertile women who conceive naturally/without ART within 6 months of ART registration have an increased likelihood of having a baby with a birth defect, perhaps due to use of fertility medication, such as for ovulation induction. The association with preterm birth for the subfertile group who take longer to conceive is similar to that seen for women who have ART.

Recruiting Men to Investigations of the Psychosocial Consequences of Male Infertility is Difficult

(Poster Presentation)

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¹Key Centre for Women's Health in Society, School of Population Health, The University of Melbourne, ²Department of Obstetrics and Gynaecology, University of Melbourne, and Melbourne IVF Reproductive Services, Royal Women's Hospital, Melbourne, Australia

Aim: The aim of this project is to investigate the reproductive, social, psychological and relationship consequences of a diagnosis of male infertility in the medium term.

Method: In 2006 a cohort of men who consulted a specialist andrologist for evaluation of infertility in 2001 and 2002 were invited to complete a postal questionnaire about their reproductive decisions and health outcomes after a diagnosis of subfertility. Men who agreed to participate were asked to return the completed questionnaire and those who did not wish to take part were asked to sign and return a form declining participation. An explanatory letter stated that unless a response was received within 2 weeks, they would be telephoned to ascertain their intention to participate or not.

Results: Of the 277 eligible participants 78 (28%) returned the completed questionnaire and 26 (9%) returned the decline to participate form within 2 weeks. The remaining 173 men were telephoned. Of these 88 stated that they were intending to complete the questionnaire but only 34 (12% of the total) actually did, yielding a total return rate of 40%. Forty-three (16%) men declined participation and 42 men were left a telephone message reminding them about the study but none of them returned the questionnaire.

Conclusion: In spite of active strategies, recruiting men to participate in research about the psychosocial consequences of infertility and its treatment is difficult. We speculate that this might reflect ongoing shame and secrecy about male factor infertility that has not been addressed in standard care.

Sperm Motility Enhancement with Low Level Laser Therapy

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Aim: To quantify the effects of Low Level Laser Therapy (LLLT) on human sperm motility in-vitro. Significant literature details the photo-bio-modulation effect of LLLT on human tissue from both diode and LED sources at wavelengths from 630 to 1000nm but no data exists on spermatozoa. The intracellular effects arise predominantly from stimulation of ATP production via the respiratory chain of the mitochondria. LLLT has been shown to temporarily improve canine sperm motility and calcium transport within murine spermatozoa.

Method: Three human semen specimens were subjected to LLLT from two continuous output sources at varied distances using the Thor-Laser system: 104 LED diode cluster of 660nm and 850nm (1.77–28.32J/cm²), and a 200mW laser 810nm GaAlAr diode (0.66–2.66J/cm²). Sperm motility of test and control aliquots was assessed using the sperm motility index (SMI) and the total functional sperm count (TFSC) parameters measured with a SQA IIB analyser.

Result: The bio-modulation from both light sources was consistent across samples with different degrees of effect. The SMI and TFSC increased up to four fold compared to controls with an inhibitory effect at higher doses. The maximum effect post-exposure varied with the light source, being the lowest dose at 30 minutes for the LED cluster and the intermediate doses at 15 minutes for the diode laser.

Conclusion: The results demonstrate that human sperm motility is modified by exposure to LLLT with an effect that is both dose and sample dependent. This justifies further study of varying sample quality at different wavelengths and source types, plus determination of the longevity of the enhancement effect and verification that it is not detrimental to DNA integrity.

How Does the Age of the Recipient Affect Pregnancy Outcome when the Oocyte Donor is Aged <35 with Proven Fertility & without, in Comparison to Those Aged >35 with Proven Fertility & without?

(Poster Presentation)

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Aim: It is thought that the age of the recipient receiving donor egg for IVF is irrelevant in relation to pregnancy outcome when

the oocyte donor is 35 years and younger. The aim of the study was to determine whether this hypothesis is true, and whether proven fertility is a contributing factor.

Method: Comparative analysis was carried out for pregnancy and live birth details from 144 recipient cycles using donated oocytes retrieved from donors age ≤ 35 and >35 , with and without proven fertility from the Melbourne IVF database and medical records between 2005 and 2007.

Results: Comparative analysis shows pregnancy rates were similar for recipients ≥ 35 using donors ≤ 35 regardless of proven fertility, whereas for recipients age <35 pregnancy rates were much higher when using donors age ≤ 35 with proven fertility (77.78%) compared with donors ≤ 35 without proven fertility (36.36%). When donors >35 were used, positive pregnancy outcomes were similar across all recipient age groups (<35 (42.86%), 35–40 (36.36%) and 41–51 (39.63%)), but only observed in recipients when the donor had proven fertility. The live birth rate was generally higher for all recipients when donors were age ≤ 35 with proven fertility (<35 (22.22%), 35–40 (20%) and 41–51 (20.69%)), compared to donors age >35 with proven fertility (<35 (14.29%), 35–40 (13.64%) and 41–51 (11.63%)). A generally poor live birth rate was seen for recipients of all ages with donors without proven fertility; regardless of age.

Conclusion: Although the contention that recipient aged is irrelevant in relation to pregnancy outcome when the oocyte donor is ≤ 35 could not be supported or disputed with the present donor data. The results do highlight oocyte donors with proven fertility in all age categories studied show a higher pregnancy and live birth rate than donors without proven fertility.

A “Snapshot” of Couples Perception of how Modifiable Lifestyle Choices Might Affect Fertility

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Aim: Modifiable lifestyle behaviours such as smoking and obesity adversely affect the chance of conceiving and delivering a healthy child (Homan et al., 2007). Despite this evidence, the lifestyle of a substantial proportion of the Australian population may be adversely affecting their general health and fertility. Methods for encouraging people to make changes to improve their health and well being are not well established.

This pilot study aimed to determine infertile couples perception of lifestyle factors affecting fertility and the chance of conceiving a healthy child.

Methods: 10 infertile couples were asked a series of structured questions regarding their understanding of the impact of some lifestyle behaviours on conception and pregnancy.

Results:

- Most couples perceived smoking and overweight to be problematic.
- Most couples thought that stress would negatively affect the chance of conception.
- All were aware of the dangers of recreational drug use.

- Most were concerned about possible effects of alcohol consumption on conception.
- While all females were taking some over the counter supplement, only half were taking folic acid.
- Although believing that overweight could be harmful to conception, some of the obese females did not think that weight was affecting their own chance of conceiving.

Conclusion: This pilot study demonstrated that couples were aware of the potential impact of some lifestyle behaviours on fertility. Implications of obesity, a significant risk factor, were not fully recognized by the obese females in this sample. While participants wanted to make lifestyle changes, common barriers included lack of time and motivation. There is a need for research and development of effective means to assist couples in making “healthier choices”.

Reference

Homan GF, Davies M, Norman R (2007). The impact of lifestyle factors on reproductive performance in the general population and those undergoing infertility treatment: a review. *Hum. Reprod. Update* 13:209–23.

Endometriosis: Infertility Demographics and Prevention

Lone HUMMELSHOJ

Publisher/Editor-in-chief, www.endometriosis.org, Secretary General, World Endometriosis Society, London, UK

Endometriosis can affect women in many different ways. For some it is pain. For some it is infertility. For some it is a myriad of associated symptoms, including fatigue, fibromyalgia, irritable bowel syndrome, compromised sexuality, chronic pelvic pain – and all the physical, mental, and financial stress associated with this, when it becomes difficult to finish an education, retain a job, maintain a relationship, and have much longed for children.

Yet little is done in terms of prevention of endometriosis. In a population of 7025 women with endometriosis an average diagnostic delay of 8 years was observed between presentation with symptoms suggestive of endometriosis and diagnosis. Less than 50% were taken serious, when they first consulted their first line practitioner, and 65% were originally mis-diagnosed with another condition, subsequently undergoing largely “hit-and-miss” treatments.

To compound this, women are not always talking openly about these issues due to the taboos surrounding menstruation, infertility, sexuality, and chronic pain.

Would the outcome be improved if we were more problem orientated vs. lesion orientated, and if these women had been treated early within a multi-disciplinary context?

Destruction of taboos and myths, increased awareness at primary level, and a more effective way to diagnose and treat endometriosis is needed to prevent unnecessary hysterectomies, to preserve fertility, to improve quality of life in women with endometriosis, and to ensure that endometriosis does not interfere with their relationships, ability to have children, finish an education or to maintain a career.

Early intervention is the only prevention.

Review of Oocyte Cryopreservation at Queensland Fertility Group 2003 to 2007

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Aim: To assess the results of 4 years experience of oocyte cryopreservation.

Method: In 4 years, 997 oocytes have been frozen and 153 thawed at QFG's laboratories. This paper reviews the results and outcomes from all thaw cycles and from cycles when all oocytes frozen have been thawed. It has been suggested that the efficiency of oocyte freezing be assessed when all the oocytes from a freeze cycle have been thawed.

Results: Post thaw oocyte survival is comparable with that of early cleavage embryos (68.0% vs 74%). An average of 5.0 oocytes were thawed per cycle (2–17) with 96.8% of cycles resulting in oocytes surviving and being suitable for ICSI.

The fertilization and degeneration rates post ICSI for both fresh (67.4%; 10.6%) and frozen oocytes (63.5%; 11.5%) are also comparable.

90.3% of oocyte thaw cycles resulted in an embryo transfer with a total of 55 embryos transferred (mean 1.9). Six clinical pregnancies resulted (implantation rate 12.7%), one of which was ectopic and a second ongoing. Five live births have occurred (one set of twins), with no abnormalities detected.

In patients where all oocytes frozen have been thawed, there is an implantation rate of 12.2%, a pregnancy rate of 28.6% per patient, and a live birth rate of 7.8% per post thaw oocyte injected.

Conclusion: These results show that oocyte freezing and thawing is a viable option for patients with moral and religious reasons against creating or freezing excess embryos and for patients with no sperm available on the day of oocyte pick-up.

Cystic Dilatation of the Rete Testis: A Hidden Diagnosis Among Infertile Men

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Aim: Cystic dilatation of the rete testis is a poorly understood condition that may be a cause of infertility in the male. It is possible that it is the result of efferent duct obstruction by epididymal cysts but other types of efferent duct anomalies and even maturational disorders of the rete have also been suggested as aetiology.

Method: This study examines the frequency of this condition among a group of 749 men attending an infertility clinic with their partners. All men underwent a testicular ultrasound whether the semen analysis was normal or abnormal.

Results: Of these 749 men, 9 were found to have this condition that was present unilaterally in 6 and bilaterally in 3 of these 9 men. The semen analysis was abnormal in 7 but normal in 2

of these 9 men. A number of other genital abnormalities were present in this group of 9 men that included epididymal cysts, testicular microlithiasis and varicoceles.

Conclusion: It is likely that this condition is indeed a cause of male infertility. However it is important that this condition is diagnosed as often as possible and this study stresses the need for the use of testicular ultrasound in the evaluation of every infertile male, even when the semen analysis is within normal limits. This lesion needs much closer examination and its role in infertility must be better defined.

Effect of Obesity on Gamete/Embryo Quality

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The prevalence of obesity in developed countries is increasing and is particularly concerning for countries like Australia and USA. There is a significant impact of obesity on reproductive function with many obese women presenting for infertility treatment due to menstrual irregularity and anovulation. Even when obese women achieve a pregnancy naturally there is a significantly increased risk of miscarriage and other pregnancy complications. Many obese women presenting for infertility treatment also have polycystic ovaries which is associated with other endocrine and metabolic disturbances that may have an intraovarian effect on the quality of the gametes produced. The medical literature is equivocal on whether obesity or at least an increased body mass index (BMI) has an effect on assisted reproduction outcomes, and if so, whether this is due to an ovarian or endometrial effect.

The Agouti mouse (Avy) is an ideal model for the study of the effect of obesity, independent of age, on gamete and embryo quality. This strain of mice is a mutant of the C57Bl/6 strain where the expression of the agouti gene controls coat colour by controlling the production of pheomelanin (yellow pigment) and inhibiting the production of eumelanin (black pigment). The Agouti gene is maternally inherited therefore Avy offspring range in colour from yellow to mottled to agouti to black (Agouti null). The yellow and mottled mice develop age related obesity and these animals can be classified as obese beyond 12 weeks of age. The agouti animals do not develop age related obesity. The black animals serve as controls on the same genetic background and these animals are lean. Obesity does not appear to effect the ovulation numbers following exogenous gonadotrophin stimulation nor the quality of the oocytes recovered as assessed by gross morphology. However as the BMI increases in the yellow/mottled mice with age the blastocyst development rate progressively decreases. This is not the case with controls where blastocyst development is similar up to 24 weeks of age when a reduction in development is age related.

The findings of an effect of obesity on in vitro embryo quality in this Avy mouse highlights the need to examine the effect of increasing BMI or WHI (waist-hip ratio) on a clearly defined population of patients presenting for infertility treatment with uniform outcome measures. In order to obtain sufficient power in these studies it is likely that this should be conducted on a national basis and could perhaps be an objective for the FSA clinical trials network.

Managing an Emerging Disease in a Threatened Species: Tasmanian Devil Facial Tumour Disease

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The emergence of new infectious diseases is increasingly being recognised as a significant threatening process in conservation biology. We present a decision tree, based on Tasmanian devil facial tumour disease, for handling novel diseases in wildlife, prioritising steps that should be taken with imperfect knowledge and that do not rely on potentially time-consuming steps of defining the aetiology of the disease or development of a vaccine or treatment.

The Tasmanian devil, formerly common and as the largest surviving marsupial carnivore an iconic species, has recently been placed on threatened species lists. A disfiguring and invariably fatal facial cancer, first reported in 1996, has now spread across most of the range of the devil, leading to population declines of up to 90% and a prognosis of likely extinction in 20–25 years. Transmission experiments have confirmed that the cancer is infectious and genetic evidence strongly suggests that it is a transmissible cell line. Limited management options include establishing isolated “insurance” populations, barriers to disease spread, and culling. Captive “insurance” populations have been established and establishing free-ranging disease-free populations on offshore islands is now being investigated.

A disease suppression trial, in which all infected animals captured are removed, is underway on a large peninsula connected to Tasmania by a bridge. Results show that the removal program has influenced the progression of the tumour epidemic. The stage structure of the tumour population has changed, with fewer large tumours now being found. In diseased populations without disease suppression, very few individuals older than 2 years are captured, whereas in this manipulated population, the age structure is similar to that of undiseased populations. The rate of geographic spread of the disease appears to have been slowed. Whether eradication can be achieved remains uncertain. We present a model structure to evaluate the effectiveness of this removal program.

Is the Cryopreservation of Sperms Before Cancer Treatment the Best Choice?

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Aim: Improved cancer treatment (CT) leads to a significant increase in survival rates. Surgery, chemotherapeutic regimens and radiation therapy are detrimental to male fertility (MF) and frequently result in infertility. Growing interest in life quality after CT has put fertility-preservation methods (FPM) into focus of physicians and patients. Approximately 75% of the patients after CT have plans for parenthood.

Method: We review available methods to maintain MF in patients undergoing CT and report experience in long time sperm storage in our Department. Retrospectively 110 cancer

patients with cryopreservation of semen (CSp) were interviewed about their acceptance for CSp.

Results: The CSp obtained before CT has become the standard-FPM employed in males. CSp give men the chance to reproduce in the future with the assistance of ART. Only 12 (7%) of males used their CSp. The reason for no using was either the protected illness, or because they do not attempt post-treatment paternity, or because they become fathers through natural conception or by using their posttreatment ejaculates for ART.

All 110 males reported a high psychological impact of CSp for the time of CT.

Conclusion: The findings give rise to emphasize two facts:

1. Only 7% of patients use their frozen semen, but the CSp has a high psychological impact during the time of CT.
2. The CSp is a powerful tool in CT of males that plans for parenthood.

Therefore, a multi disciplinary co-operation with oncologists, urologists or andrologists need to discuss male fertility preservation before CT.

Are There Non-Invasive Markers In Human Oocytes That Can Predict Pregnancy Outcome?

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Aim: To analyse pregnancy producing oocytes to find potential markers for their selection.

Method: A retrospective study of oocyte parameters using the PolScope was undertaken in patients having ICSI. 22 oocytes (19 patients) identified as forming high quality embryos that resulted in a clinical pregnancy were compared with 55 oocytes (27 patients) that formed high quality embryos (as defined by freezing of other embryos in that cycle) but did not result in a pregnancy. The parameters examined were: spindle presence, normality and retardance. Day 2 embryos were analysed for: cell number, fragmentation <10%, zona pellucida thickness (ZPT) and retardance (ZPR). Data were compared by Chi-square and student t-test depending on the nature of the variable.

Results: Detection of normally shaped spindles was significantly higher in the pregnant group (100% vs. 31%, $P < 0.001$), as was the retardance of these normally shaped spindles ($3.0 \pm 1.23 \text{ nm}$ vs. $2.4 \pm 0.7 \text{ nm}$, $P = 0.03$). Presence of an identifiable spindle at 40h post trigger was higher but not significant in the pregnant group ($n = 19 \text{ pts}$) than the non-pregnant group ($n = 27 \text{ pts}$) (100% vs. 91%, $P > 0.05$). In the analysis of the embryos, both groups had similar rates of <10% fragmentation (100% vs. 96%, $P > 0.05$). ZPT ($20.7 \pm 3.0 \mu\text{m}$ vs. $17.6 \pm 3.8 \mu\text{m}$, $P > 0.05$) and ZPR ($2.6 \pm 0.96 \mu\text{m}$ vs. $3.0 \pm 0.7 \mu\text{m}$, $P = 0.4$) were similar in both groups.

Conclusion: These data demonstrate an association between the occurrence of a clinical pregnancy and the presence of a

normal spindle and raise the possibility that detection of a normal-shaped spindle may be a useful tool in selection of a single embryo for transfer.

Adolescents' Views On Communicating About Donor-Assisted Conception

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Aim: Prompted by legislation in Victoria, Australia, permitting gamete donors to seek identifying details of people conceived from their gametes, this research investigated the views of adolescents from the general population on how parents can best talk to their donor-conceived adolescent children about their conception.

Method: Qualitative interviews (6 groups, 1 individual) with 25 secondary school students.

Results: Naïve adolescents had views largely consistent with those of donor-conceived adults, for example urging parental honesty, adaptation to individual children, and family cohesion. They identified the social father as the parent while acknowledging the significance of genetic connection to the donor. A minority asserted a preference for non-disclosure; all said that, if disclosure occurred, it should be by parents.

Conclusion: This small study contributes to increasing understanding of communication within families about donor-assisted conception.

Stem Cells to Germ Cells (Male and Female)

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Removal of leukaemia inhibitory factor (LIF) and supporting feeder cells initiates differentiation in mouse ES cells into several lineages including the germ line as evident by the expression of germ cell markers. These findings raised the hopes that in the near future stem cell therapy could be used to treat infertility related to germ cell deficiency. The requirements for germ cells to differentiate into a female or a male germ line are different and these differences should be taken into consideration when attempting in-vitro differentiation. It has been established that mouse ES cells (male or female) develop into oogonia that enter meiosis and recruit adjacent cells to form follicle like structures without any external stimuli. ES cell-derived-oocytes are surrounded by oestradiol-secreting supporting cells, which are recruited, probably, through the activation of Fig α . Nonetheless, ES cell derived oocytes do not progress through normal meiosis in-vitro. This suggests that either ES cells fail to initiate the specialized meiotic cell division program unique to germ cell in-vivo, even though they express genes related to meiosis, or that the putative ovaries have impaired physical organisation.

A sporadic appearance of oocytes in ES cells prolonged cultures raised the question if addition of growth factors, known to support ovarian development in-vivo, will support this differentiation. We examined the addition of conditioned medium obtained from new born testicular cultures to EBs and identified a repeatable way of producing ovarian like structures containing oocytes. The conditioned medium obtained from cultures of new born male testes known to contain an array of growth factors, presumably supporting this development. The oocytes obtained expressed specific markers and also were positive to SPC3 expression indicating that they undergo meiosis. Yet it needs to be identified if these oocytes are functional in embryonic development. Attempts to differentiate ES cells to functional sperm were more successful. Investigators were able to differentiate ES cells in-vivo and in-vitro to male gametes that can generate offspring in mice, after transplantation into the testis or long term exposure to retinoic acid (RA, Nayernia et al., 2006). Although most of the offspring died between 5 days to 5 months from birth due to epigenetic problems, it is encouraging progress towards achieving viable male gametes in-vitro.

Fertilisation and Development of In-Vitro Matured Metaphase I Oocytes

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Aim: To evaluate the developmental consequences of delayed in-vivo maturation and subsequent completion of maturation in vitro in human IVF oocytes.

Methods: A prospective analysis of 262 consecutive ICSI cycles. Aspirated oocytes were assessed for initial stage of maturity (GV, MI or MII) and incubated for 3–6 hours prior to microinjection. Fertilisation rate, incidence of early (23hpi) cleavage, frequency of cleavage failure and developmental rate were compared for oocytes which had completed meiotic maturation in vivo and those which underwent the MI to MII transition within 6 hours of in vitro incubation. Statistical analysis was by χ^2 .

Results: From a total of 2343 aspirated oocytes, 1820 (78%) were at the MII stage and 230 (10%) still had a visible germinal vesicle. The remaining 293 (13%) had no visible GV but had not extruded the first polar body and were assumed to be at the MI stage. After in vitro incubation, 177 of the 293 MI oocytes (60%) had extruded a first polar body (MI→MII) and underwent ICSI together with the in-vivo matured (MII) oocytes. The fertilisation rate was significantly reduced in the MI→MII oocytes (57% versus 72%; $p < 0.05$) as was the frequency of early cleavage (3% versus 15%; $p < 0.01$). Significantly more fertilised MI→MII oocytes arrested prior to the first cleavage division (16% versus 3%) but there was no significant difference in the developmental stage on day 2 between the groups.

Conclusion: The yield and quality of developing embryos is significantly reduced in oocytes which do not attain the MII stage prior to follicular aspiration.

Relationship Between Hyperactivation of Capacitated Human Sperm and Sperm-zona Pellucida (ZP) Binding and the ZP-induced Acrosome Reaction

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Aim: This study was to determine the relationship between human sperm hyperactivation (HA), sperm-zona pellucida (ZP) binding and the ZP-induced acrosome reaction (AR) in vitro.

Methods: Sperm samples from 129 infertile men were studied. Semen analysis was performed using standard methods. Motile sperm (2×10^6) selected by PureSperm were incubated with 4 oocytes in 1 ml human tubal fluid (HTF) supplemented with 10% human serum. After 2h incubation, the number of sperm bound to the ZP and the AR of ZP-bound sperm were examined. Velocities and HA of sperm after 2h incubation in the same medium were assessed by Hamilton-Thorn Sperm Analyzer (IVOS 10).

Results: The HA was highly correlated with the ZP-induced AR (Spearman $r = 0.626$, $P < 0.001$) but not with sperm-ZP binding ($r = 0.019$, $P > 0.05$). Men with $<7\%$ HA sperm were more likely to have very low ZP-induced AR. Only normal sperm morphology was significantly correlated with sperm-ZP binding ($r = 0.346$, $P < 0.001$). Sperm motility and velocities were significantly correlated with sperm morphology but not with either sperm-ZP binding or the ZP-induced AR.

Conclusion: That HA correlates only with the ZP-induced AR and not with sperm-ZP binding suggests a mechanistic link between HA and the physiological AR in humans. Both HA and the ZP-induced AR may be required for sperm to penetrate the ZP. Assessment of HA of capacitated sperm in vitro may be a useful clinical test for male infertility associated with defective ZP-induced AR that does not require the use of human oocytes.

Day 3 SET – Which Embryo Do You Choose?

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Aim: To refine embryo selection to identify those with the highest implantation potential. This will be helpful in single embryo transfer cycles (SET) with several embryos of similar quality.

Method: Retrospective analysis of Fertility Associates clinics since 2003. All patients under 39 with SET (N = 1465). Analysis included development at 25 hrs post insemination (early cleavage), cell number on day 2 and cell number on day 3. Implantation Rates (IR) were compared within a D2 vs D3 development matrix for each of 3 early cleavage types.

Results: Overall, all embryos that were 2PN at 25 hrs had a lower IR (37%) than the syngamy (46.8%) and cleavage group (48.7%). However, there was no difference in IR between embryos that were 2PN (48%), syngamy (51%) or cleaved

(54%) providing they were 4 cells on day 2 and 8 cells on day 3. There was a binomial distribution of IR in 2PN embryos against cell number on day 2 and day 3 with a peak at 4 cells on day 2 and 8–9 cell on day 3. D2 and D3 cell numbers were less important if 25h embryos were in syngamy or cleaved.

Conclusion: Embryo scoring is a multifactorial process. IR rates for some 'types' of embryo were higher or lower than we would have expected from previously published systems. This exercise was useful as part of the evolution of the embryo selection protocols for our labs.

Abstract withdrawn

Prevalence and Significance of Mononucleated Blastomeres in Human 4 Cell Embryos on Day 2 of Development

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Aim: To assess the distribution of blastomeres with single observable nuclei in human 4 cell embryos on day 2 and its relationship to early developmental markers of viability.

Methods: Normally fertilised (2PN) oocytes from ICSI or conventional IVF cycles were assessed for timing of entry into syngamy and the first cleavage division at 23/24 hours post insemination (hpi). Embryos which subsequently contained 4 blastomeres at approximately 42 hpi (n = 628) were scored for visible nuclei. Statistical analysis was by χ^2 .

Results: Nineteen embryos (3%) contained multinucleated blastomeres and were excluded from further analysis. Of the 609 remaining embryos, 370 (61%) had a visible nucleus in all four blastomeres, 51 (8%) in three blastomeres, 95 (16%) in two blastomeres, 14 (1%) in one blastomere and 79 (13%) had no visible nuclei. The distribution was equivalent for embryos generated by ICSI and conventional IVF. Of the embryos which had entered syngamy by 23/24 hpi, 72.4% (326/450) had four visible nuclei at 42 hpi compared to only 27.7% (44/159) of embryos which had not undergone nuclear envelope breakdown (NEBD) by 23/24 hpi ($p < 0.001$). In contrast, only 4.8% (22/450) of the embryos which had entered syngamy by 23/24 hpi had no visible nuclei at 42 hpi compared to 35.8% (57/159) of embryos which had not undergone NEBD by 23/24 hpi ($p < 0.001$).

Conclusion: Presence of a visible nucleus in all blastomeres of a 4 cell embryo at 42 hpi is related to timing of entry into syngamy, an established predictor of subsequent implantation potential.

Pregnancy in Women with Polycystic Ovarian Syndrome Attending a Lifestyle Modification Group (Poster Presentation)

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Aim: Polycystic ovarian syndrome (PCOS) is an endocrine condition, where many patients exhibit insulin resistance which can lead to weight gain, and a disturbance of ovulatory function and infertility¹. The infertility and menstrual problems for this group of women have traditionally been treated with hormones, rather than addressing the broader health issues for women with PCOS. Studies report weight loss, achieved in a group program, consisting of healthy eating and exercise, leads to an increase rate of spontaneous ovulation and pregnancy², thus a lifestyle modification program was set up to ascertain if these lifestyle changes could assist our overweight PCOS patients.

Method: Overweight women with PCOS attending Endocrine/Metabolic Services outpatient clinic were enrolled to participate in a 4 month program comprising of 5 hours of educational/self development and exercise sessions each week. Clinical and biochemical evaluations were conducted at the beginning and conclusion of the program.

Results: 303/383 (79%) women completed the program; with 62% wanting conception. Only 4.9% women were ovulating at the beginning of the program, with the remainder being oligo/anovulatory. At the completion of the program 55% of the women were ovulating. Of the women still wishing to conceive

at the completion of the program 67.8% have done so to date, and 53.4% of these have been spontaneous. The remaining 46.6% of pregnancies went on to use some form of assisted reproductive technology.

Conclusion: These results demonstrate that lifestyle modification is an effective approach, and should be considered as the first line treatment for overweight women with infertility associated with PCOS.

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A Four Year Survey of Chromosome Abnormalities in Males Presenting for the Investigation of Infertility

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Aim: To investigate the frequency and type of chromosome abnormalities in males presenting for infertility investigation over a 4 year period and to correlate this with their semen parameters.

Method: All cases with a chromosome abnormality had semen parameters and clinical indications for investigation collated.

Results: A total of 4930 males were karyotyped and 85 (1.75%) had a chromosome abnormality, either a balanced rearrangement or a numerical abnormality. Robertsonian translocations occurred in 17.6% of abnormal cases, 46.6% having abnormal semen parameters; reciprocal translocations occurred in 30.5% of abnormal cases and 50% had abnormal semen parameters; inversions occurred in 9.4% of abnormal cases with no abnormal semen parameters; sex chromosome abnormalities (including numerical, structural and mosaicism) occurred in 31.7% of abnormal cases and 92% had abnormal semen parameters; extra small accessory chromosomes (ESAC) occurred in 3.5% of abnormal cases and 33.3% of these showed abnormal semen parameters; insertions were found in 1.2% of abnormal cases with no abnormal semen parameters; and unusual variants were found in 5.8% of abnormal cases with 25% showing abnormal semen parameters. Translocations (Robertsonian and reciprocal) account for half the structural rearrangements observed. In the sex chromosome group, 77.7% had a 47,XXY karyotype. Of note in this group, sperm were found in one 47,XXY patient and in one sex reversed patient. Only 8% of the abnormalities were previously known to the patients.

Conclusions: Karyotyping is indicated in the clinical work up of all male patients presenting with infertility to allow appropriate counselling and for clinical strategies to be developed to maximise the chance of a normal pregnancy.

Is Clinical Pregnancy Loss Increased with Obesity?

(Poster Presentation)

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Aim: It is believed obesity increases miscarriage or spontaneous abortion (SAB) rates. The aim of this study was examine pregnancy loss in obese women being treated for infertility.

Methods: Outcomes of pregnancy in 209 obese women either enrolled in a "big girls group" (151) or known to have a BMI >25 or weight greater than 80kg were compared with those of other patients treated at the same time. While all had been counselled to reduce weight before conceiving most were still overweight at the time of treatment.

Result: There were 3 SAB in 28 clinical (ultrasound sac(s) positive) pregnancies (11%) for 381 nonART treatments: ovulation induction or artificial insemination with partner or donor sperm. There were 19 SAB in 91 pregnancies (21%) from 489 fresh embryo transfers from IVF or ICSI and 21 in 98 (21%) pregnancies from 483 frozen embryo transfers (FET). There were also 2 ectopic pregnancies in the FET group. The SAB rates were lower if only first pregnancies were analysed: nonART 8%, IVF/ICSI 12%, FET 17%. For all patients treated by ART between 1993 and 2006 the pregnancy loss rate was 21%.

Conclusion: These data do not support the contention miscarriage rates are higher in obese women.

"Who Needs Sex Anymore? We're on IVF!" – The Importance of Assessing and Addressing the Sexual Health of Patients Presenting for ART

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Aim: This paper will explore and highlight the importance of promoting broader sexual health to patients undergoing ART treatment procedures.

Method: This is a review paper and will present a range of empirical data to support the aim.

Results: It is commonly acknowledged that the onset of 'sex for conception' changes the way a couple think about sex, and also the role that sex plays in their relationship. When sex is no longer 'just for fun' a couple's sex life can change dramatically. Often, there will be an initial rise in intensity and excitement due to the freedom from contraception and the desire to procreate. However, with a continued lack of success in being able to conceive naturally, couples' sex lives can become regimented and dramatically impaired – and this is often the case by the time they present for ART treatments.

Conclusion: Sexual health has recently been prioritized as an area of people's lives that requires 'protecting, supporting and restoring' and a healthy, satisfying sex life is a key element of successful relationships. Sex and fertility are intrinsically linked, yet surprisingly little attention is focused on assessing and addressing the sexual health of patients experiencing fertility

problems. Sexual dysfunction may be a contributing factor to fertility problems in some couples, and in others dysfunction may develop following difficulty conceiving. Sexual distress may also be present within the relationship and have implications for the overall functioning of that relationship.

While we now have medical interventions which allow for conception without sexual intercourse, we cannot forget the need to address our patients' sexual health. Sexual problems affect a large proportion of the population and yet often go undetected and untreated. This paper will highlight that counsellors working in ART treatment centres are ideally positioned to assess and detect sexual health issues both before starting and during ART so that they can be addressed through either onsite counselling or appropriate referral.

Fertility in Males: Strategies Across Life

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Childhood

Undescended testes are strongly linked to infertility and cancer. Surgical correction may reduce the risk but it persists probably due to an underlying developmental disorder.

Adolescence and adulthood

Vascular: Testicular torsion, inguino-scrotal surgery, blunt trauma or intra-tunical arterial bleeding (e.g. from surgical sperm retrieval) may result in atrophy.

Infective: Acute epididymo-orchitis may cause obstruction or permanent spermatogenic damage underscoring the need for safe sex practices. Mumps orchitis is prevented by vaccination.

Heat: Immersion in hot water transiently impairs sperm output. Studies reporting reduced semen quality in some occupations are often confounded by design flaws. Data on 'tight underwear' are conflicting. Severe febrile illness transiently reduces semen quality.

Drugs: Opiates and sex steroids inhibit gonadotropin secretion: recovery is likely but often delayed.

Chronic or severe illness suppresses serum testosterone levels and spermatogenesis.

Sexual dysfunction: Disease (e.g. diabetes) or surgical damage may result in erectile/ejaculatory problems.

Ageing: Reduced semen volume & sperm motility occurs in healthy aging while other evidence suggests impaired sperm DNA integrity. Age-related reductions in male gamete quality are modest in comparison to the female. Confounding factors may partly account for increased time-to-pregnancy and reduced ART outcomes in older men.

Cancer at all ages

Chemotherapeutic damage to spermatogonia is drug and dose-related. Alkylating agents are the greatest culprits but less gonadotoxic regimens are being developed. Low dose radiation effects are transient but permanent azoospermia may occur with fractionated doses over 2Gy. Sperm cryopreservation must be offered: ICSI allows fertility despite poor semen

quality. Gonadal protection strategies have been disappointing. Spontaneous recovery usually occurs within the first 3 years. In persistently azoospermic men, surgical sperm retrieval for ICSI is successful in 50%. Data on offspring of long term cancer survivors exposed to a range of such treatments are re-assuring.

Fertility Preservation in Hematology Cancer Patients

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The treatment of hematological malignancies often poses a threat to female fertility. Various strategies to preserve fertility are commonly practiced before commencing potentially sterilizing cancer treatment. These include in-vitro fertilization (IVF) and cryopreservation of ovarian tissue. Over the last 14 years fertility preservation procedures were performed in hematology female cancer patients.

Patients' hematology records and fertility consultations charts were evaluated, IVF laboratory results and surgical reports were revised and ovarian histology was investigated. Our data clearly demonstrates that chemotherapy protocol and the urgency for chemotherapy initiation, patient's general condition and the partner's status determines fertility preservation procedure chosen. Previous cancer treatment and especially short interval from chemotherapy to ovarian stimulation influence IVF outcome. Recent animal study reassures that ovarian stimulation for IVF before chemotherapy administration does not adversely effects ovarian reserve compared with identical cytotoxic treatment to unstimulated ovaries.

With ovarian tissue cryopreservation investigation included medical and endocrine evaluation, preoperative imaging, laparoscopic inspection and histological evaluation of fresh ovarian tissue. Partial oophorectomy was the preferred surgical procedure. With recent reports of pregnancies and deliveries after transplantation of thawed ovarian tissue more cured cancer patients will request re-implantation of stored ovarian tissue. To increase procedure safety, identification of small number of cancer cells in ovarian tissue is indicated. After thawing histology, immunohistochemical staining and most sensitive recently developed molecular markers were used however, their clinical significance is still under investigation. Medical complications associated with previous cytotoxic treatments and radiotherapy might have remote adverse effect. Therefore, fertility treatments and pregnancies in cured cancer patients should be carefully monitored. The optimal approach for fertility preservation varies, and should be individualized according to clinical judgment and previous experience.

Daughter, Sister, Mother? Issues Surrounding Daughter to Mother Egg Donation

Catherine NAVE

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The use of family members as donors can be a very satisfying experience for all involved, as well as enabling families to main-

tain genetic linkages to the offspring. As acceptance of 3rd party reproduction continues to develop, so too do expectations of having individual choice over the form in which these collaborative arrangements should take. One such arrangement is daughter to mother egg donation.

There is extremely limited data about these arrangements, which usually occur when the mother is in a 'second marriage'. Although the daughter who is donating may be an adult, by the very nature of these arrangements she is usually young, thus can the potential for influence and coercion ever be overcome? Counselling in relation to egg donation explores the boundaries and potential relationships for all involved, particularly for the child. Although there are many and varied family formations in existence, is the boundary too blurred in these circumstances?

This paper explores the issues and concerns in relation to daughter to mother egg donation and whether these situations should be undertaken at all, and if so on what basis?

Antral Follicle Count by Three-Dimensional Inversion Mode Ultrasonography and Ovarian Power Doppler Angiography may Predict Ovarian Response and IVF/ICSI Outcome

(Poster Presentation)

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Aim: To evaluate whether three-dimensional ultrasonography (3D-US) and power Doppler angiography (PDA) measurements may be useful tools to predict ovarian response during controlled ovarian hyperstimulation and/or are associated with IVF/ICSI outcome.

Method: Prospective clinical study in 83 women undergoing IVF cycles settled in a tertiary care teaching hospital. A long standard protocol with pituitary suppression was used. Ovarian volume (OV), number of antral follicles >5mm (NAF) and PDA indices: vascularisation index (VI), flow index (FI), and vascularisation flow index (VFI) were evaluated by 3D-US inversion mode and PDA on the day of pituitary suppression control. OV, NAF, PDA, age and BMI were correlated with the number of follicles >18mm on the hCG day and the number of MII oocytes retrieved. Statistical analysis was performed with SPSS software, version 11.5. Analysis of variance (ANOVA), Mann-Whitney U-test, χ^2 and Fisher's exact test, Pearson method and Multiple regression analysis were used to compare categorical data, where appropriate. A P-value <0.05 was taken as being significant.

Results: OV, NAF and PDA indices correlate significantly ($P < 0.01$) with the number of follicles >18mm and MII oocytes recovered. OV and the NAF predicted significantly the number of recruited follicles ($R = 0.71$) and MII oocytes retrieved ($R = 0.69$). The number of MII oocytes, Grade 1 embryos and the pregnancy rate were higher if the NAF was ≥ 6 .

Conclusions: 3D-US inversion mode and PDA are useful tools to predict ovarian response. OV and NAF predict the number of follicles developed and MII oocytes retrieved.

A Review of the Fertility Centre's Semen Storage*(Poster Presentation)*

Bettina NEWSOME

The Fertility Centre, 249 Papanui Road, Christchurch, New Zealand

Aim: As New Zealand is well known for having one of the world's highest vasectomy rates we wanted to assess when and why people freeze samples, and how the samples were used.

Method: The Fertility Centre has been providing a semen storage service since March 1993. Data from the database was analysed.

Results: Between March 1993 and March 2007 we have frozen 788 samples for 499 clients. The average number of samples a person froze is 1.6. This service has evolved into freezing for several categories:

Categories	Samples frozen	No. of men	Age range (years)	Couples using samples	Pregnancies
Pre-vasectomy	414	238	20.8–61.5	5	1
Treatment back-up	184	139	25.6–61.8	10	7
Vas reversals	82	56	32.1–56.0	5	2
Post surgical sperm	27	25	27.0–61.3	3	0
Pre-medication	27	15	18.2–40.9	2	0
Personal	26	12	22.4–47.1	2	0
Pre-surgery	19	11	18.7–65.2	0	0
Medication	9	3	28.6–38	0	0

Of the samples used, 71% were used within 12 months. Other samples were discarded on average after 3.3 years. Overall, only a small percentage have come back to use the samples they froze. From 788 samples, 73 were used (9.2%). We have had 10 live births, 7 from treatment back-up samples. 12% of men have stored samples for more than 10 years.

Conclusion: Considerable time and effort is involved in providing this service, however we consider that if it gives our patients a little peace of mind for future treatments or options later in life it is worthwhile.

Empty Cots and Silent Spring in an Age of Plenty – What Our Lifestyle Is Doing To Our Reproductive HealthProfessor Robert NORMAN^{1,2}¹Director Research Centre for Reproductive Health, University of Adelaide, ²Repromed, Adelaide, Australia

Australia and New Zealand's birth rates have fluctuated considerably over the last century and exhibit striking influences of war, famine, social and economic deprivation and prosperity. Some of this can be ascribed to medical contraception but much of the changes in the first half of the twentieth century are due to socio-political-behavioural changes. After the devastation of the First World War, many young women were widows or unable to find husbands with a resultant drop in births for

several years. Since the 1960s we have seen a sustained reduction in birth rates which, even with the slight increase in the last 3 years, shows an ongoing downward trend.

Fifty years ago, Rachel Carson published her book *Silent Spring* showing how environmental chemicals such as DDT have reduced wildlife populations and now we face allegations of environmental pollutants affecting sperm counts. Over and under nutrition, smoking, delayed childbearing, single lifestyle and many other factors are impacting on our ability to reproduce when we want to have children. Evidence-based data on the true factors affecting our birth rate are lacking but we should have enough information to act now.

I will cover diet and nutrition as an example of how we are affected by both environmental factors and our own choices. There is abundant data to suggest adverse influences of obesity on natural reproduction and pregnancy and for lifestyle interventions to improve fertility. Assisted reproduction may reverse the adverse anti-fertility effects of obesity but merely passes the problem on to another group of medical colleagues in pregnancy. Smoking is also highly anti-fertility and should be stopped before pregnancy. Other agents will also be covered.

Clinics dealing with infertility in Australia and New Zealand must face the issues of lifestyle modification as treatment prior to fertility management. Patients should be aware of the risks they face by continuing their adverse lifestyle habits. Governments should have policies to help couples conceive earlier and in an economic and social environment where they are optimally healthy for pregnancy. The Fertility Society has an important role to play but individuals must also change their practice and advice to reduce intervention and promote optimal fertility.

Acknowledgement

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Chlamydia Surveillance – Why Worry?

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Genital infections with *Chlamydia trachomatis* are now the commonest notifiable disease in Australia. This is predominantly an epidemic in young people under the age of 26 years. Infections in women are notified at least twice as often in men. Chlamydia infections are a common cause of ectopic infection, tubal infertility and pre term delivery. Vertical transmission is also common with the result of increased middle ear infections, conjunctivitis and pneumonia in infants.

For over 10 years the state of Tasmania has conducted enhanced surveillance for each case of Chlamydia. The results of this surveillance activity will be presented and used to highlight the risk factors for acquisition of Chlamydia infections as well as to determine which people should undergo routine and regular screening.

Islamic View of Gamete and Embryo Donation or Surrogacy

(Poster Presentation)

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Aim: So far, there are too many children are born by donation protocols or surrogacy. In Islamic countries upon the third conference of the Islamic Fiqh Council in 1986, all kinds of donations are banned. So, no Islamic country practices donations except Iran in which, all of them are practiced everyday. As Iran represents an Islamic country and most of the Iranian people are Muslims, there can be some social, legal and psychological concerns for the children born by these protocols that should be considered, discussed and clarified for avoiding future complications. This study outlines some proofs and documents for helping the Islamic law-makers, clergy leaders and professionals to pursue a practical guideline in this regard and maybe an upgrade to the previous statement.

Method: As we can not find the equivalent terms in the Islamic resource, we review the Islamic resources mostly the Holy Quran to find some proofs and we could find out the Islamic ideas.

Results: (1) These protocols are compared with adultery in many papers and statements but it has been shown that they are totally different. (2) It has been stated that these protocols bring confusion of lineage that does not. (3) Some relations like intimacy have been discussed that is OK in these families. (4) Two vision of cell donation and multiple marriages have been discussed and proved that cell donation vision is accepted and no fake marriage is OK in Islam. (5) We suggest two-mother theory that clarifies these children lineage so other rights linked to the lineage upon Islam. (6) We underline the anonymity of the donor to avoid complication that may come if the donor identity is known, that upon Islam donors will be true parents also, and some rights goes back to them. (7) We have suggestions about the inheritance of the children born by sperm and embryo donation which can not be solved by ordinary Islamic law.

Conclusion: It has been shown that Islam can accept donation and surrogacy by presenting proofs and documents from Islamic resources and clergy leaders can pursue a practical guideline in this regard.

Outcomes of Follicular Reduction (FR) at Stimulated Intrauterine Insemination (SIUI) in a Nested Case Controlled Study

(Poster Presentation)

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Aim: Our primary objective was to compare the pregnancy rate (singleton and multiple) of SIUI cycles where women underwent FR to that of controls.

Method: Data was collected for all SIUI cycles between August 2000 and December 2005. Selective FR was performed

on patients who have over-responded (more than three follicles 14mm in size) defined by local protocol. Each case of FR was matched with two randomly selected controls. Statistical analysis was performed using chi-square and with $p < 0.05$ considered significant. Of the 520 patients who underwent SIUI during this period, 27 had FR and 54 were the selected matched controls.

Results: There was no statistical difference in the age, day 2 FSH, total FSH used, number of follicles before SIUI and pregnancy rate between the FR and control groups. There was one twin pregnancy in the control group and none in the FR group.

Conclusion: These results suggests that follicular reduction in SIUI treatment in patients who have over-responded is both effective and avoids cycle cancellation.

Medical Management of Ectopic Pregnancy with Extremely High Beta-hCG Levels Following In Vitro Fertilisation (IVF)

(Poster Presentation)

Kee J ONG, James BROOKER

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Aim: A case of medical management of ectopic pregnancy with beta-hCG level of 85 230 iu/L.

Method: A 32 years old women presented with ectopic pregnancy after in vitro Fertilisation. At laparoscopy, she was found to have a frozen pelvis due to her severe endometriosis. No obvious ectopic pregnancy was identified. Decision was made to treat the patient medically. She received multiple dose regimen of methotrexate and was treated as an inpatient.

Results: Ectopic pregnancy was successfully treated but patient experienced some pleuritic chest pain and took 3 months for serum beta-hCG to become undetectable. Patient also complained of painful vaginal bleeding for 2 weeks whilst being treated as an inpatient.

Conclusion: In cases where surgery is risky, medical management of ectopic pregnancy is possible even with extremely high beta-hCG. However, treatment to resolution time can be prolonged and it is associated with side effects.

Supercool: Blastocyst Vitrification

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Aim: The aim of this study was to compare the outcomes of vitrification and slow freezing for the cryopreservation of blastocysts.

Method: Frozen embryo transfer cycles performed in 2007 using blastocysts that had undergone slow freezing (generated in 2006) or vitrification (2007) were retrospectively analysed. Blastocyst freeze criteria included embryos that displayed a blastocoel cavity, inner cell mass and adequate trophoctoderm on day 5 or 6 of culture. Vitrification involved the use of a double cryoprotectant DMSO/ethylene glycol

with the cryologic vitrification kit. Slow freezing was performed using a glycerol based commercial blastocyst freezing kit.

Results:

Rates	Vitrification	Slow Freezing
Survival	92/104 (89%) ^a	178/218 (82%) ^a
Preg/Warming	37/78 (47%) ^b	34/148 (23%) ^c
Preg/Transfer	37/72 (51%) ^d	34/126 (25%) ^e
FH/Embryo transferred	24/58 (41%) ^f	29/162 (18%) ^g

FH = Fetal heartbeat at 7 weeks

Rows with different superscripts differ significantly by chi-squared analysis ($p < 0.01$). Blastocysts cryopreserved using vitrification showed a significantly higher pregnancy and implantation rate compared to slow freezing. Blastocyst survival rates were comparable.

Conclusion: Vitrification is a highly successful means of storing blastocysts and preliminary results show the procedure to be more successful than slow freezing.

The Midbody Controls Cleavage Furrow Formation during Meiosis in Mouse Oocytes

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Aim: The midbody consists of a mass of granules that develop in the equatorial region of the spindle during the anaphase and telophase of mitosis. Plk1 has been reported to localize to the midbody during meiosis in mouse oocytes. However, the role and composition of midbody structures in meiosis require to be elucidated. Our approach was to examine the midbody by immunocytochemical studies and the interaction of the meiotic structures by time-lapse DIC microscopy.

Method and Results: Three-dimensional confocal laser microscopy showed that a component of the meiotic apparatus consisted of numerous dot-like particles that were aligned on the plane of the spindle's plate-like surface. When mouse GV oocytes were treated with cytochalasin B, chromosome segregation occurred and midbody-particles appeared but the space between each particle did not narrow. Subsequently, the midbody degraded gradually and polar body extrusion failed. Interestingly, to outward appearances, the telophase oocytes reverted back to metaphase. The same situation occurred when the midbody was disrupted by laser. This reversal was characterized by dissolution of the midbody, disappearance of the cleavage furrow, and realignment of chromosomes on the metaphase plate. When a MI spindle was trapped, parallel to the surface, just before the onset of chromosomal segregation, a cleavage furrow opened toward the midbody and divided the oocyte into 2-cells, instead of forming a polar body. Subsequently, MII spindles were formed in each of the cleaved cells.

Conclusion: Our experimental data suggest that contraction of the midbody was associated with actin polymerization and may also be directly involved in cleavage furrow formation.

Vitrification Using Fibreplug™ Does not Impair Development of Mouse Embryos

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Aim: To use mouse embryo vitrification as a model for training clinical embryologists.

Method: A total of 313 mouse (F1: C57/Bl6 × CBA) embryos were fertilized in vivo ($n = 194$, collected at 2-cell at 40-hrs post-hGG), and in vitro ($n = 119$, oocytes collected 17-hrs post-hCG). The embryos were generated and vitrified by Master of Clinical Embryology students at Monash University from the 2007 intake. Embryos were either vitrified (day 3 or 4, $n = 258$), warmed and then cultured to day 5 or cultured for 5 days (controls, $n = 55$). The CVM1™ system (Cryologic-Australia) was used for vitrification. Individual embryos were picked up in 2–3 µL vitrification media (15% EG, 15% DMSO and 0.6M sucrose in KSOM-Hepes) supplemented with 0.3% or 1% BSA and transferred onto a hook (Fibreplug™). The Fibreplug™ were then touched onto a pre-cooled metal block sitting in liquid nitrogen then inserted into a pre-cooled straw, thus avoiding direct exposure of embryos to liquid nitrogen. Data were analysed using χ^2 -test.

Results: A total of 236 embryos were recovered from 245 warmings (3.7% loss). All treatments were combined as no differences were detected between embryo source, day vitrified and BSA content. After warming, 195 embryos (82.6%) survived and developed to blastocysts which was not significantly different to controls (89% reached the blastocyst stage).

Conclusion: From these data, we suggest that vitrification by the CVM1™ system does not impair embryo development and is a technique easily learned and performed by students in the early stages of their training program.

Embryo Donation: To Give or Not To Give, That is the Question

(Poster Presentation)

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Aim: In New Zealand, embryo donation to other couples is a novel procedure, and one that has not been taken up by many. Embryo donation for research purposes is currently under review. Given its novelty, this study explores how potential donors and recipients in New Zealand make meaning of embryo donation.

Method: Twenty potential donors and potential recipients of embryos were interviewed regarding their thoughts around

embryo donation. Data was analysed qualitatively using a discourse analytic approach to identify the ways in which people represent embryo donation.

Results: Our findings show the contextual and ambiguous nature of decision-making in relation to embryo donation. Our participants' views on embryo donation depended in part on the meaning they associated with the embryo (as life, collections of cells, potential children, something in between), as well as how the practise of embryo donation is carried out in New Zealand and the perceived implications thereof.

Conclusion: Embryo donation remains surrounded by controversy and is a complex, often emotionally and ethically problematic issue for those in the position to donate or seeking to be recipients.

A Comparison of English Speaking and Non-English Speaking Female Monash IVF Patients: An Exploratory Analysis

Sarah PHILLIPS, Celia GONCALVES

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Aim: The current study was aimed to explore the overall differences between non-English speaking patients (NESP) and English speaking patients (ESP) in IVF presentation and outcomes over an 11-year period. Ultimately, it is anticipated that an understanding of these differences will then lead to the development of an informed Culturally Sensitive Practice to address any differing needs of NESP.

Method: Retrospective data analysis was performed on ESP and NESP who completed a standard IVF or donor mandatory counselling session between 1995 and 2006 inclusive. A total of 11,523 cases were identified, in which 11,324 were ESP and 322 were NESP.

Results: Independent samples t-tests revealed significant differences between the two groups; female NESP were older than ESP at the time of presenting to Monash IVF, and completed fewer ART cycles overall. NESP also had significantly fewer ART pregnancies and ART live births, and also fewer non-ART live births from their current relationship. Additionally, there were 33 languages spoken by NESP, with Vietnamese and Mandarin being the most frequent. Finally, there were significantly more NESP presenting to Monash IVF over time.

Conclusion: Reasons for why female NESP are older than female ESP at the time they present for ART, and why they complete fewer ART cycles, need further investigation. Currently, opportunities exist for the translation of fundamental information, such as fact sheets, into Vietnamese and Mandarin. Furthermore, due to the increasing frequency of NESP over time it is essential that a Culturally Sensitive Practice inclusive of a Cultural Plan is developed.

Mothers' and Fathers' Experiences of Complicated Childbirth: Implications for Couples Considering ART Multiple Pregnancies

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Aim: The proportion of critically ill infants is rising due to advances in medical technology such as assisted reproductive technology (ART) and improvements in obstetric care, allowing infants to survive at younger gestational ages. The aim of this study was to qualitatively investigate mothers and fathers' entire experiences of complicated childbirth in order to identify more appropriate avenues for intervention and support at all stages.

Method: A phenomenological study was undertaken comprising of fifty one-hour interviews with 25 couples that had experienced a complicated childbirth, such as very early premature birth. Issues explored included communication with medical staff, involvement in decisions regarding treatment and care, support, and associated emotional responses.

Results: Results showed that both parents desired to be treated as autonomous individuals and to have their decisions respected. While the primary concern of both mothers and fathers was for the survival and long-term health of their infant, they reported markedly different family roles and support needs. Fathers felt excluded and insignificant in the care for their newborn, whilst mothers felt enormous pressure to develop physical and emotional bonds. Mothers also experienced a wider range of negative emotional responses to their complicated childbirth experience, which often lead to long-term implications for the nuclear family system.

Conclusion: Avenues for improved support and interventions for couples throughout pregnancy and childbirth were identified, including strategies for communication and practical interventions for both parents and hospital staff. Implications for counselling couples considering multiple pregnancies through ART are also identified and discussed.

Nursing Challenges Presented by Preimplantation Genetic Diagnosis Patients

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Preimplantation Genetic Diagnosis (PGD) has been a possibility for many years, and is increasingly being offered by IVF clinics. It can be presented as a simple option for those wanting a family unaffected by a particular genetic condition. In reality though, the undertaking of PGD for genetic reasons can highlight many existing physical and social issues.

This paper draws on the experience of nursing in a PGD programme and identifies cycle management and counseling issues which differ from the usual infertility patient. It is important for IVF nurses to have some understanding of particular conditions and whether there are specific issues for carriers, those affected and/or potential offspring.

PGD patients usually have a background of a severe medical condition which actually or potentially affects them, family

members or their own children. Each may have faced issues of loss, chronic illness, or inheritance factors, which may have impacting family issues including blame, guilt and secrecy. All will have had to consider options for having a family with a range of ethical, social, financial and physical implications. For some, the genetic condition may make for more difficult IVF stimulation or have variable side effects.

There are also emotional and practical issues associated with having normal fertility and being on an infertility programme, which can also impact on a persons ability to cope with PGD.

This paper will look at PGD in general but will specifically focus on Huntingtons Disease which forms our biggest group of referrals.

Association of Long Polyglycine Tracts (GGN repeats) in Exon 1 of the Androgen Receptor Gene with Cryptorchidism and Penile Hypospadias in Iranian Patients

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Aim: To determine whether changes in the polyglycine tracts in exon 1 of the androgen receptor gene are associated with Cryptorchidism and Penile Hypospadias in Iranian Patients.

Method: We performed a comprehensive case-control study of 76 cryptorchid and 92 hypospadiac (divided into subgroups of glanular, penile, and penoscrotal hypospadias) Iranian males. The length of the CAG/GGN repeat segment was evaluated by using PCR-sequencing in exon 1 of AR gene. To eliminate other mutations in the AR gene, exons 2–8 of AR gene were screened by PCR-SSCP.

Results: There were no significant differences in CAG lengths between the cases and controls but GGN numbers were found to be significantly higher (median 24 vs. 22) among both subjects with penile hypospadias ($P = 0.018$) and those with a history of cryptorchidism ($P = 0.001$), compared with controls. In addition, the GGN numbers among subjects with penile hypospadias were significantly different, compared with the two other subgroups of hypospadias ($P = 0.001$). We were able to identify 12 different CAG alleles and 8 different GGN alleles in the cryptorchid group.

Conclusion: The mean GGN repeat length increased with the cryptorchidism and penile hypospadias. However, statistical analysis showed no significant differences between the cryptorchidism subgroups (bilateral or unilateral) ($P > 0.05$). The distribution of GGN allele frequencies was different between cryptorchid men and controls and there was an apparent trend toward a shift to GGN = 22 or GGN > 22 in males with cryptorchidism, but no significant difference was observed with hypospadiac group respect to controls.

Distinct Spectrum of CFTR Mutations and IVS8 (TG)M(T)N Variants in Iranian Males with Congenital Bi/Unilateral Absence of the Vas Deferens

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Aim: Study the involvement of the CFTR gene mutations and polymorphisms in CBAVD and CUAVD in Iranian population with presumed low cystic fibrosis (CF) frequency and finding CFTR gene mutation spectrum for Iranian patients.

Method: We analyzed CFTR mutations and (TG)m(T)n polymorphism in 112 Iranian CBAVD and 7 CUAVD males from Iran with 84 fertile males as control. Moreover, we compared the rate of decrease in exon 9+ transcripts with reduction of the (T)n repeat in our studied population.

Results: Forty-six of the 112 patients with CBAVD (41.07 percent) had two mutations in the CFTR gene, 41 of them had the 5T allele (in 11 cases two allele of 5T was detected). Forty-three patients (38.39 percent) had a mutation in one copy of CFTR gene in witch 9 of them had just one 5T allele. IVS8-5T was observed with TG12 or TG13 haplotypes, on 61 chromosomes thus confirming the association of this splice site variant with CBAVD in Iranian patients. The F508del mutation in exon 10 was uncovered on 28 chromosomes, thereby revealing high frequency of this mutation in Iranian CBAVDs. Screening for the IVS8-5T and F508del together led to the identification of more than one-third of alleles. Exon 9 skipping was strongly joined with 5T/5T genotype, the rate of normal CFTR mRNA increased by having IVS8-9T (TG)₉₋₁₀ and IVS8-7T (TG)₁₀₋₁₁₋₁₂. We could detect one novel nonsense mutation (K536X) in the NBD1 region and two novel missense mutations (Y122H & T338A) in the M2 and M6 regions of CFTR gene in our studied population witch were not reported previously. The Y122H and T338A mutations were compound heterozygote with IVS8-5T. The conservation of changed nucleotide and amino acid in mutated regions were analyzed by aligning with nine different species. We also observed the highest level of the exon 9+ splicing efficiency between the tested samples with the TG12T7 allele.

Conclusion: Our study showed that the 5T mutation revealed a high frequency in our patients. The longer of (TG)m tract increases the proportion of exon 9– transcripts, but only when activated by the 5T allele. So our results support the idea that a putative role of the (T)n repeat is to distance the TGm repeat from the 30 splice site, and the different alleles at the Tn locus affect the efficiency by which the splice acceptor consensus sequence is recognized.

Natural Cycle IVF (In Vitro Fertilization) – An Analysis At Our Centre

(Poster Presentation)

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Aim: We evaluated the outcome of natural cycle IVF in patients with a poor ovarian response or a raised follicle stimulating hormone (FSH).

Materials and Methods: This was a retrospective case analysis of 16 natural cycles in 11 IVF patients. All patients were stimulated with clomiphene if the FSH was raised. Follicular growth was monitored using ultrasound and hormonal evaluation. Oocyte retrieval was scheduled following a natural LH surge or human chorionic gonadotropin (HCG) administration. All statistics were done using SPSS version 14.0.

Results: The mean age of the patients was 36.5 years (range 29–41). We had 3 pregnancies in 16 cycles. There were oocytes recovered in 67% of the cycles and 42% had an embryo transfer. Clomiphene citrate stimulation was associated with oocyte recovery in 73% of the patients compared to 40% without the drug (Fisher's exact test 0.299). There was oocyte recovery in 71.4% of patients with a natural LH surge. Oocytes were retrieved in 78% of individuals when the FSH was over 10 U/L and in 86% when the day 2 estradiol was more than 80 pmol/L. The pregnancy rate per cycle was 19% with 2 of the patients being less than 35 years old.

Conclusions: We recommend clomiphene stimulation in natural cycle IVF to maximise oocyte retrieval. Serum FSH and estradiol are not predictive of the ovarian response. Spontaneous LH surge can be used to time oocyte recovery. This is a reasonable option for a young poor responder before considering oocyte donation or adoption.

Proteomic Analysis of the Endometrium on the Day of OPU

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Aim: At oocyte pick-up (OPU) the endometrium is more advanced than in natural cycles and this may negatively impact on implantation. Our aim was to identify protein patterns associated with poor implantation. This could assist in developing protocols that more optimally prepare the endometrium for embryo transfer.

Method: Endometrial biopsies were obtained at OPU. In experiment 1 (Exp.1) biopsies from fertile oocyte donors (n = 4) were compared with those from IVF patients with primary infertility and more than 4 failed IVF transfers (n = 4). In Exp.2 biopsies from a separate group of fertile oocyte donors (n = 4) were compared with those from IVF patients with idiopathic infertility (n = 4).

Proteins were extracted, labelled with fluorescent CyDyes, focused on a pH 3–10 gradient, and subjected to 2D SDS PAGE on large 24 cm gels, which were scanned and analysed using SameSpots software.

Results: The comparison in Exp. 1 revealed 61 protein spots that were significantly different (p < 0.01 for 10 spots), 30 of these were selected as strong candidates for further investigation. Exp. 2 revealed 41 protein spots that were significantly different (p < 0.01 for 8 spots), 19 of them were selected for further study. Further analysis has revealed that a number of those significant spots are the same in Exp.1 and Exp.2. Current work predicts a larger number of significant differences among less abundant, but potentially important proteins with increased resolution of protein separation.

Conclusion: Proteomics is a powerful technology that may allow us to identify which protein patterns predict a favourable environment for implantation.

NK Cell Analysis in Women with Recurrent Reproductive Failure: There is a Clinical Correlation Between Peripheral Blood and Endometrial Biopsy Samples

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Introduction: It is hypothesised that excessive natural killer (NK) cell activity is a cause of persistent reproductive failure. The value of peripheral blood testing is unknown, in part because it has been argued that blood NK cells are unrelated to uterine (u)NK cells, and hence there is no scientific rationale for testing them. However, blood testing would be a far less invasive and hence better option for patients. This study aimed to assess whether there is a correlation between blood and uterine NK cells.

Materials and Methods: Women with repeated miscarriage or IVF failure were recruited (n = 29). Blood samples were analysed by flow cytometry and, based on previous clinical publications, NK cell numbers were stratified as 'normal' (<12%), 'borderline' (12–18%) and 'high' (>18%). Endometrial biopsy samples analysed by immunohistochemistry were similarly stratified as 'normal' (<5%) and 'high' (>5%) uNK cell levels.

Results: In the luteal phase, the median peripheral blood NK count was 13.32% of lymphocytes (4.8–26.45) with a mean concentration of $0.28 \times 10^9/L$ (0.05–0.59). uNK cell density had a median of 3.5% (1–58).

8 women had 'high' blood NK levels, and 5 of them (71%) had elevated uNK levels. 5 women had 'borderline' blood levels and 3 (60%) had high uNK levels. And of the 16 women with 'low' blood levels, only 6 (37%) had elevated uNK levels. There was a strong correlation between blood NK and endometrial NK samples (Pearson correlation co-efficient p < 0.01).

Conclusions: This is the first study to demonstrate a correlation between peripheral blood and uterine NK cells and thus provides some validity for peripheral blood testing in the assessment of persistent reproductive failure.

Factors Influencing Reproductive Health Risk Taking Behaviour in University Students

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Aim: To investigate the factors that influence reproductive health risk taking behaviour utilizing the Theory of Reasoned Action (TRA) and Social Learning Theory (SLT).

Method: A self completed questionnaire was administered to students attending the University of Western Australia. Seventy males and 107 females (mean age 21.5 years, SD = 2.98) characterized themselves on demographic, experiential and attitudinal variables relating to fertility risk behaviours (delaying pregnancy, substance use, sexual behaviour, weight). Logistic regression was used to determine significant predictors of engaging in fertility risk behaviour using components of TRA and SLT.

Results: The majority of men and women (59.9%) intended to delay childbearing to a point where female fertility is diminished (≥ 30 years). Participants who intended to delay childbearing were significantly more likely to perceive ART as a viable option to overcome fertility problems compared with people who did not intend to delay to this point ($p = 0.035$). Logistic regression showed that participants who were more aware of the adverse effects of advanced maternal age on fertility were more inclined to report intentions to delay childbearing compared to those who do not ($p = 0.012$). Normative beliefs were predictors of smoking and substance use, however, they were not strong predictors of childbearing intentions, sexual behaviour or weight status.

Conclusion: Although university students had acceptable levels of knowledge of fertility risk factors these were not strong predictors of their fertility risk taking behaviour. The focus of a 'fertility protection' campaign should centre on education, empowerment and providing transparent information on the availability and utility of reproductive services.

Day 12 to 16 Serum Progesterone and hCG as Predictors of Pregnancy Outcome Following ART

(Poster Presentation)

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Aim: To investigate the relative value of serum progesterone and hCG, measured 12–16 days after ovulation as predictors of viable pregnancy following ART treatment.

Method: Logistic regression was used to examine the value of day 12–16 progesterone and hCG to predict viable pregnancy.

We used data from 2811 treatment cycles at Concept Fertility Centre, Subiaco WA, between January 1994 and December 2004. Inclusion criteria were: treatment with IUI, IVF, ICSI or FET; serum hCG and progesterone measured 12–16 days post-ovulation; and pregnancy outcome recorded. Viable pregnancies were defined as birth of alive fetus ($n = 1546$). Non-viable pregnancies ($n = 1265$) included biochemical pregnancy, spontaneous abortion and ectopic pregnancy.

Results: Women with a viable pregnancy were significantly younger than those with a non-viable pregnancy ($p < 0.001$) and had significantly higher day 12–16 progesterone and hCG ($p < 0.001$). Outcome of pregnancy did not differ by treatment type. The regression model containing age and hCG correctly predicted 74.6% of non-viable pregnancies and 87.6% of viable pregnancies with an overall correct discrimination of 81.7%. Including progesterone marginally improved the ability of the model to classify non-viable pregnancies (75.4%).

Conclusion: Day 12–16 serum levels of hCG combined with patient age can be used to predict the likelihood of viable pregnancy following different types of ART treatment. Measurement of progesterone is of little clinical utility once hCG level is taken into account.

Expressing our Disquiet – Counsellors and Embryo Donation

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Aim: Embryo donation is a treatment with the potential to raise ethical and emotional issues for the donors, recipient couples and staff involved. ANZICA counsellors wished to have a position paper to represent their views on embryo donation, this paper will trace this tricky task.

Method: Through a series of presentations, emails and debates the issues of embryo donation were raised multiple times for counsellors. Finally, the first of many drafts of the position paper were presented and the real debate began by phone and email.

Results: Engagement was tricky and initially slow but as the emotional awareness of the paper, as representative of ANZICA counsellors, increased so too did the speed with which responses arrived. Thorough debate took place and a consensus of sorts was reached.

Conclusion: ANZICA counsellors have expressed their concerns but recognize they still have to work with embryo donation within their clinics. The position paper was approved by the ANZICA executive in April 2007 and distributed to the members.

Spermatogonia in the Primate Testis: A Target for Male Fertility Preservation?

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Aim: We have targeted spermatogonial stem cells in the non-human primate testis. It was our goal to characterize stem cells

and progenitors and to describe patterns of premeiotic germ cell expansion. We then explored options to maintain and mature immature monkey tissue via xenografting and explored various gonadotoxic exposures as well as preservation strategies on xenograft survival and developmental potential.

Method: We have applied organ cultures and xenografting of immature monkey tissue. Nude mice were used as recipients. We have analyzed Bouin-fixed adult and immature testes which were embedded in resin or paraffin. We also described cellular aggregates in whole mounts of seminiferous tubules. BrdU-incorporation was used for detection of proliferating cells.

Results: The monkey testis contains stem cells and self-renewing progenitors. Spermatogenesis starts with synchronous divisions of 16 A_{pale} spermatogonia arranged as pairs or quadruplets at stage 7 of the seminiferous epithelial cycle. A second division of spermatogonia occurs at stage IX which is characterized by a doubling of cells and clones, but stable number of cells in each clone. The subsequent divisions of B-spermatogonia lead to clonal enlargements until after several more divisions large cohorts of preleptotene spermatocytes are formed. A dark spermatogonia show labeling indices below 1% and may act as reserve stem cells. Xenografting of juvenile rhesus monkey testicular tissue into nude mice induced testicular maturation. At 3–4 months first spermatocytes are encountered. Effects of irradiation or gonadotoxic treatment can be studied in xenografts after exposure of the testicular tissue prior to grafting or treatment of the recipients. Short term storage of juvenile tissue on ice for 24 hours or cryopreservation using DMSO as protectant was possible without losing the ability of xenografts to initiate development.

Conclusion: Primate testicular stem cells are excellent targets for preservation of the male germline. The presence of abundant spermatogonial stem cells and self-renewing progenitors allows an easy isolation of cells. Organ culture and xenografting are excellent strategies to explore spermatogonial physiology. Initiation of spermatogenesis in xenografts offers novel strategies to generate primate sperm and might open scenarios for clinical applications.

The Need for a Nursing Role in Caring for Patients Who Have Ovarian Hyperstimulation Syndrome

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Aim: To conduct a needs and quality analysis of clients who had been hospitalised with Ovarian Hyperstimulation Syndrome (OHSS) in order to inform the creation of a responsive and evidence based nursing role to cater for these clients.

Method: Participants were recruited from patients admitted to Royal Women's Hospital under Melbourne IVF for OHSS between 2003 and 2006. A survey was created and sent out with a covering letter to potential participants, there was a 61% (69/113) return rate on the patient surveys. Data was analysed using the Statistical Package for the Social Science version 12.0 (SPSSv12). Analyses included frequency data analysis and correlation analyses.

Results: Results showed a statistically significant relationship ($p < 0.05$ level) between the length of in hospital stay and length

of disability, but not between High Dependency Unit (HDU) admission and length of disability. Patient education about OHSS before commencement of treatment was found to be 59.4% and 66.7% after OHSS diagnosis. Satisfaction with nursing care showed that overwhelmingly patients were very satisfied (73.9%) with only 4.3% of the participants recording dissatisfaction.

Areas highlighted for improvement were methods of education, information topics, and nursing intervention. Types of nursing intervention that were most desired were follow up phone calls at home post discharge, visits from the IVF nurse whilst an inpatient, and detailed written information.

Conclusion: The data collected strongly suggests an important role for an OHSS liaison nurse in the education of patients undergoing IVF treatment and the staff involved in patient care.

A Comparison of the Development of ICSI Embryos Resulting from Fresh and Frozen Oocytes

(Poster Presentation)

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Aim: To compare the growth and development of ICSI origin embryos from fresh and frozen-thawed oocytes.

Method: The early cleavage rate, embryo development and morphological grades of over 60 embryos resulting from ICSI fertilized thawed oocytes were compared with the development of over 18,000 ICSI embryos from fresh oocytes.

Results: There were more pronuclear oocytes at the 24 hour check in the frozen oocytes compared with the fresh oocytes (85.7% v 56.3%), with less frozen oocytes in syngamy (8.6% v 27.7%) and showing early cleavage (5.7% v 15.7%).

The day two development showed that there were more non-cleaved oocytes in frozen oocyte cycles than in fresh oocyte cycles (27.9% v 5.5%). There were more 2-cell and 3-cell embryos on the morning of day 2 in the frozen oocyte group than in the fresh oocyte group (32.8% v 20.9%) and (13.1% v 11.4%). There were less 4-cell and 5-cell or greater embryos in the frozen oocyte group compared with the freshly injected oocytes (23% v 50.1%) and (3.3% v 12.1%).

The morphological grades of day 2 embryos from fresh and frozen oocytes were: very poor quality embryos (10.1% v 27.9%); poor quality embryos (18.8% v 11.5%); average quality embryos (37.8% v 41.0%); above average embryos (23.8% v 16.4%) and excellent embryos (8.6% v 3.2%).

Conclusion: The results indicate that there the frozen oocyte group had poorer developmental markers (early cleavage, day two development stage and day two morphology) than injected fresh oocytes.

Review of a Variable Blastocyst Transfer Policy – A Fence Sitters Perspective

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Adopting a variable blastocyst culture approach with an opt out option for cases with poor embryo development resulted in no

cancelled transfers and a pregnancy per egg collection as good or better than routine day 3 transfers when frozen transfers are included. Such a policy allows clinicians to encourage patients to plan for day 5 transfers with the reassurance that all couples will have a transfer and that a pregnancy is achieved with the fewest transfers.

Aim: Many clinics are interested in blastocyst transfer but are concerned about poor cryosurvival and potential cancelled transfers. One option is to adopt a mixed transfer policy including rescheduling day 5 transfers to day 3 where required. The options include committing all embryos to day 5, limiting embryo number to day 5 with the remainder frozen on day 3 or transfer and freeze on day 3. This review is to assess whether a mixed day 5 transfer strategy is viable compared to routine day 3 transfers when conceptions from both fresh and frozen transfers are combined.

Method: All cases in 2006 were included in the study. The decision to proceed to blastocyst culture was patient based and part of the consent process. Various culture media were employed for blastocyst culture and all embryos were frozen using slow freezing protocols. Single embryo transfers were standard for women under 35 years of age on initial cycles otherwise up to 2 embryos were transferred regardless of age.

Results: Of 387 couples proceeding to embryo transfer in 2006, 71 proceeded to day 5 transfer. There were no cancelled day 5 transfers due to poor blastocyst quality. The fresh clinical pregnancy rate (CP) for women less than 40 years of age was 35% for day 3 transfers (average 1.2 embryos per transfer) and 43% for day 5 transfers (average 1.1 embryos per transfer). Value adding to this rate with subsequent frozen embryo transfers increased the cumulative pregnancies per egg collection to 53% for day 3 and 68% for day 5 transfers. The number of additional frozen transfers per fresh transfer was the same in both groups (1.6 total transfers per egg collection). For women over 40, day 3 transfers generated more pregnancies from both fresh and frozen transfers (18% day 3; 8% day 5).

Conclusions: Adopting a policy of selected day 5 transfers with an opt-out clause generated a similar or better productivity in terms of clinical pregnancies per egg collection and fresh transfer to that of a 3 protocol where the woman was aged under 40 years. More pregnancies were obtained by proceeding to day 5 than when embryo transfer was performed on day 3 but less pregnancies were generated from frozen embryos. This data review argues that both models generate a similar net number of pregnancies per collection and that where embryo numbers and quality permit, opting for extended culture is a viable management process since a pregnancy may be achieved sooner rather than later. Our data did not support blastocyst culture for women over 40 years.

Sperm Morphology Audit – Assessment of Individual Spermatozoa Reveals Worrying Skill Level

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A survey of 126 individual spermatozoa over 2 years by between 35 and 93 participants of an online quality assurance program indicated a wide variation in the grading of each spermatozoon as normal or abnormal. This survey revealed only

27% uniformity in assessment and highlighted an underlying uncertainty in interpretation of morphology definitions and argues for specific training and certification in this critical skill.

Aim: To survey the assessment of individual spermatozoa to understand the high variation in samples returned from quality control audits.

Method: QAPonline provides a monthly quality control program to individual scientists involved in Andrology. One assessment task was to mark individual spermatozoa as either normal or abnormal within the WHO Sperm Morphology Modules. The number of participants who completed these tasks varied between 32 and 93 each month. The results from this audit between 2005 and 2007 covered 126 spermatozoa (between 3 and 5 per month). Each was graded by the degree of agreement between participants on whether each spermatozoon was normal. If the agreement was between 0 and 10%, the spermatozoa were deemed abnormal and if the agreement was between 90% and 100% the spermatozoa were deemed normal.

Results: 29 spermatozoa were deemed abnormal and only 6 deemed normal in this audit. Thus only 27% of spermatozoa appear to be able to be assessed with a reasonable degree of certainty while 63% of spermatozoa were unable to be defined with any degree of uniformity. Thirty of the 126 spermatozoa were surveyed on the various components of the sperm for assessment. Assessment of the head, and to a lesser extent, the midpiece morphology reflected the same variation as the primary audit while the tail, cytoplasmic droplet and the acrosome status indicated far better uniformity.

Conclusion: The audit on the grading of individual spermatozoa revealed poor uniformity of identifying whether each sperm was normal or abnormal. Ancillary questions implied that the interpretation of the head and midpiece morphology was the primary area of confusion. While many medical and scientific personnel pay lip service to sperm morphology results, such diversity in visual skills should be seen with some concern since it is this component of a semen analysis that is most likely to encourage clinical referral to ICSI. Such diversity would not be acceptable in other areas of pathology eg cervical screening, and suggest that fertility bodies could consider further training and certification before scientists are permitted to report this characteristic.

Lifestyle Factors Affecting Pregnancy Rates

(Poster Presentation)

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Aims: To report which lifestyle factors affect fertility as measured by time to pregnancy (TTP) in a population of pregnant women (16–32 weeks) with planned pregnancies who participated in a WHO surveillance study of TTP and semen quality.

Method: Women pregnant between 16 and 32 weeks were asked to complete a questionnaire on TTP. Eligible male partners (18–50 years, he and his mother Australian born, n = 225) were asked to undergo a physical examination, complete addi-

tional questionnaires and provide blood and semen. Factors affecting pregnancy rate were analysed by Cox regression.

Results: From 2061 couples who completed TTP questionnaires (response rate 98%) there were 928 eligible males of whom 225 (24%) participated. Of the 2061 women, 74.3% had planned pregnancies, 11.6% had contraceptive failures, 9.5% had other unplanned pregnancies and 4.6% were treated for infertility. For planned pregnancies, pregnancy rate was negatively related ($p < 0.05$) to female smoking around time of conception and illicit drug use. There was no significant effect on pregnancy rate of self-reported exposure to noxious agents either in females or in males. No significant differences in semen characteristics between male smokers and non-smokers were found.

Conclusions: Lifestyle factors in the female (smoking and illicit drug use) reduce pregnancy rates but there were no significant effects via the male.

National Survey of Cryopreserved Embryos in Storage at Australian Fertility Clinics

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Aim: To determine the number and disposition of cryopreserved embryos in storage in fertility clinics in Australia.

Method: A national survey of fertility clinics was conducted in 2006. Clinics were asked to provide summary data on the number of cryopreserved embryos in storage at the time of survey implementation. Data were also collected on length of storage and disposition. Disposition was categorized as: patient treatment, donation to other couple, donation to research, lost contact, awaiting disposal, and other. Of 65 eligible clinics, 58 (89.2%) responded with 57 (87.7%) providing data on number of cryopreserved embryos.

Results: 118,709 embryos were in cryostorage in 57 clinics. Disposition of 105,109 embryos ($n = 52$ clinics) were flagged as: future patient treatment (90.2%), donation to research (5.6%), awaiting disposal (2.7%), donation to other couple (1.0%) and lost contact (0.1%). Period of cryostorage was available for 117,117 embryos ($n = 55$ clinics) with 45.1% in cryostorage for ≤ 2 years and 24.7% for > 5 years. Most embryos for future patient use (51.2%) were in storage for ≤ 2 years compared to > 5 years for donation to either research at clinics with NHMRC research licences (77.0%) or to other couples (61.5%).

Conclusion: Most cryopreserved embryos in storage in Australia are for future patient use with few flagged for donation which is consistent with the literature.¹⁻³ While national data is currently limited, fertility clinics have the capacity to monitor embryo storage and disposition at a treatment and patient level. Enhanced reporting at a national level can be achieved through the development of ANZARD.

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Vitrification with Fibre Plugs; Efficient, Easy to Learn and Suitable for Cryopreserving Cleavage Stage Embryos

(Poster Presentation)

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Aim: Cryopreservation of human embryos has traditionally utilized the slow freezing methodology. After several years experimental work in the vitrification of embryos in animal species, there has been a slow and steady adoption of the technique clinically, but mainly at the blastocyst stage. We have utilized both in vivo and in vitro produced 2 cell mouse embryos to test whether this technique is suitable for cleavage stage cryopreservation and if easily adopted by trainees ($n = 3$) with less than 4 months embryo handling experience. We also compared 2 different protein levels (0.3% and 1% BSA) in the KSOM Hepes media, which was the base to the vitrification and the warming media.

Method: Individual embryos were picked up in 2.5 uL vitrification medium, (15% ethylene glycol, 15% DMSO and 0.6M sucrose in KSOM-Hepes), placed on fibreplugs and vitrified using the Cryologic Vitrification Method (CVM1 kitTM).

Results: Nine embryos from 142 warmings were not found and a further 12 embryos did not survive the thaw. The percentage of embryos reaching the blastocyst stage from those that survived thawing was similar to unvitrified controls for in vivo produced vitrified embryos (55/56, 98% vs 10/10, 100% respectively, Chi-squared test), and in vitro produced vitrified embryos (44/55, 80% vs 10/12, 83% respectively). BSA concentration had no effect on the developmental potential of embryos in either group.

Conclusion: From these data, we suggest that vitrification is an easily adopted technique suitable for vitrifying at the early cleavage stages.

Comparison of the Use of Ejaculated and TESA/Biopsy Sperm in ART for Patients with High Levels of Sperm DNA Fragmentation

(Poster Presentation)

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Aim: The aim of this study was to compare the outcomes of cycles using ejaculated and TESA/biopsy sperm in cases of high sperm DNA fragmentation to determine the best source of sperm for treatment in these cases.

Method: The outcomes of 40 treatment cycles, including IUI, IVF, ICSI and FET cycles, that took place at Fertility First in 2006 were analysed retrospectively. All couples in which the male had been found to have a sperm DNA fragmentation level of 20% or higher prior to cycle commencement were included. Sperm DNA fragmentation was determined using the terminal uridine deoxynucleotidyl transferase-mediated nick-end labelling (TUNEL) assay. Spermatozoa were obtained from the testicles using either needle aspiration or open biopsy technique under local anaesthetic.

Results: The clinical pregnancy rate per cycle for women aged <40 years and using TESA/biopsy sperm (53%) was significantly greater than that achieved using ejaculated sperm (0%) ($\chi^2 = 7.84$, $P < 0.01$). The clinical pregnancy rate per cycle for women aged ≥ 40 years and using TESA/biopsy sperm (9%) was also greater than those using ejaculated sperm (0%), however this difference was not significant.

Conclusion: These results suggest that in this setting, men with a sperm DNA fragmentation level of 20% or higher are much more likely to achieve a pregnancy if they provide TESA/biopsy sperm for treatment. This information will prove valuable in improving both patient care and outcome of ART. Further investigation of larger samples will be necessary to confirm the reference value of 20% demonstrated here.

A Novel Assay for Identification of Oxidative Stress Related Male Infertility

(Poster Presentation)

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Aim: Oxidative stress related damage to sperm has been reported to affect up to 75% of men in infertile relationships. Existing chemiluminescent assays require expensive equipment not readily available in clinical andrology laboratories. Therefore, most ART units currently do not test for sperm oxidative stress. As antioxidant therapies have now been shown to boost male fertility there is a need to develop a sensitive, rapid and economic assay for sperm oxidative stress.

Method: The NBT assay is a well established test of somatic cell oxidative stress but has not been standardized for use in sperm. The generation of superoxide anions within the sperm cytoplasm results in oxidation of NBT to diformazan, which is then quantified on a standard ELISA plate reader. The diformazan product per million sperm was compared between 12 fertile and 31 infertile men, as well as being correlated with markers of sperm oxidative damage (TUNEL, membrane expression of annexin V and lipid peroxidation by products).

Results: A NBT result of 45?M (mean + 2 SD of fertile controls) was identified as a good cut-off point separating fertile from infertile men in relation to free radical generation (sensitivity 77%, specificity 92%). This NBT cut-off point also proved useful in differentiating between healthy and damaged sperm.

	Fertile (n = 12)	Infertile-low NBT (n = 7)	Infertile-high NBT (n = 24)
Tunel (%)	10.2 ± 4	11.8 ± 3.1	23.9 ± 18
Annexin V (%)	9.9 ± 5	13.9 ± 4	24.6 ± 13
NBT (?M)	22.9 ± 11.0	18.9 ± 8.7	210.3 ± 362
LPO-586 (MDA units per 10 ⁶ sperm)	0.36 ± 0.27	0.31 ± 0.20	7.37 ± 16.73

Conclusion: The NBT test is an easy to perform assay which helps identify infertile men with oxidative stress related damage to their sperm.

Is There a Significant Difference in Ovarian Response between Oocyte Donors <35 with Proven Fertility & Without, in Comparison to Those >35 with Proven Fertility and Without?

(Poster Presentation)

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Aim: At Melbourne IVF, the donor program aims to treat recipients using oocyte donors between the ages of 25–40 years who have completed their family, as it is widely reported that a woman's fertility declines from the age of 35 onwards. There is limited information otherwise however, on what other details can be used as a benchmark when looking for a donor, e.g. proven fertility. Thus the aim of this study was to examine the details of donor cycles, particularly successful cycles to establish if proven fertility should also be a consideration when looking for a donor.

Method: Comparative analysis was carried out on ovarian response (in terms of number of follicles produced, number of eggs retrieved and number of embryos formed) collected from 144 oocyte donor cycles from the Melbourne IVF database and medical records between 2005 and 2007.

Results: Recruitment rate was found to be the greatest in donors >35 age group with proven fertility 74%, compared with the ≤ 35 years age group without proven fertility who overall produced the least amount of follicles. Egg retrieval rates and embryo formation rates were similar across all age categories irrespective of proven fertility.

Conclusion: These findings indicate that proven fertility should be another factor to consider when choosing a donor.

Age-Specific Success Rates for Women Undertaking Assisted Reproduction Technology Treatment Using Fresh Autologous Oocytes, Australia 2002–2004

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Aim: To determine the pregnancy and live birth rates for women in 1 year age increments following initiated assisted reproductive technologies (ART) cycles.

Method: A retrospective population-based study of 54,594 cycles conducted in Australia during 2002–2004 of women using fresh autologous oocytes. Pregnancy and live birth rates per oocyte pick-up were determined based on 1 year increments in women's age (age at treatment) with grouped age at the extremes. Pregnancy outcomes were adjusted for type of infertility. Logistic models were built for women aged ≥ 30 years to predict the success rates.

Results: The mean age of women was 35.3 (range 17–51) years. The overall live birth rate was 19.3%. Male factor only infertility had a slightly higher live birth rate (20.8%) than female factor only infertility (17.7%). The highest success rates were among women aged 27 years with advancing maternal age associated with a decline in success rates. For women ≥ 30 years each additional 1 year in age, was associated with an 11% (99% CI: 11%–12%) reduction in the chance to achieve pregnancy and 13% (99% CI: 13%–14%) reduction in live births. Compared to women aged 25–29 years, women ≥ 43 years were 8.5 (99% CI: 6.9–10.4) times less likely to get pregnancy.

Conclusion: These crude rates for pregnancy and live births provide a good indicator of the likely success based on women's age at treatment. Advancing women's age from 30 years onwards is associated with incremental decline in success rates. Women aged ≤ 41 years achieved a reasonable live-birth rate of $>5\%$.

Open Testicular Biopsy Outcomes at Monash IVF from 2005–2006

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Aim: To investigate sperm retrieval rates and ART outcomes for men undertaking open testicular biopsy at Monash IVF for 2005–2006.

Method: A retrospective analysis of results of consecutive open testicular biopsies performed at Monash IVF from 2005 to 2006, including data on success at sperm retrieval, pregnancy and live birth rates.

Results: Open testicular biopsies were performed on 42 men undertaking a total of 48 IVF cycles. 21 patients had failed sperm retrieval at PESA or FNA, while 21 progressed straight

to open testicular biopsy. The patients included 5 men with Klinefelters, 2 Yq microdeletions, 18 primary spermatogenic failure (STF), 7 cases of cryptorchidism, 2 men with chemotherapy-induced STF, 1 with STF secondary to mumps orchitis, and 1 with structural (axonemal) sperm defects. Five men with obstructive azoospermia required open biopsy to retrieve sperm (1.2% of all men presenting with obstructive azoospermia). In 22/42 (52%) of cases, sperm was retrieved from open biopsy and used for ICSI. In 20/42 (48%) of cases, no sperm was found, with 75% of those with no sperm at open biopsy using a donor back-up. Of the 22 men with sperm retrieved, 12 pregnancies (55%), and 10 live births (45%) were achieved (7 singletons, and 3 twin-sets). This was a successful birth rate with their own sperm for 24% of the patients starting a cycle with open-biopsy in this series.

Conclusion: We report favourable outcomes for men requiring open testicular biopsy to retrieve sperm for injection at ICSI. Patients need to be counselled accurately about the chances of success with surgical sperm retrieval versus donor sperm before embarking on IVF treatment, according to aetiology.

Laparoscopic Instrument Insulation Failure: The Hidden Hazard

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Aim: The aim of this study was to determine the prevalence of insulation failure in gynaecological laparoscopic instruments with a secondary aim of assessing the impact of routine static insulation failure testing.

Method: A cross sectional survey (Canadian Task Force Classification: II-2) of insulation failure in laparoscopic operative instruments was conducted in two public tertiary teaching hospitals. The main outcome measure was the dichotomous assessment of instrument insulation failure and the characterisation of insulation defects.

Results: One hundred and eleven instruments were tested. The overall prevalence of insulation failure was 27% with a rate of 39% in dedicated monopolar instruments. The sensitivity of visual inspection to predict a damaged instrument was 10%. Even when the site of the failure was identified, the defect was only detectable in 35% of instruments without magnification. The mean site of insulation failure was at 71 mm from the tip of the instrument, placing the majority of insulation defects within the abdominopelvic cavity during surgery. Following the introduction of routine static electro-surgical instrument testing, the overall prevalence of insulation failure dropped to 5.9%.

Conclusion: There is an unacceptably high prevalence of instrument insulation failure in gynaecological laparoscopic instruments. Visual inspection is not an appropriate screening mechanism for insulation failure but routine biomedical testing reduces the prevalence of defective laparoscopic instruments.

Sperm Chromatin Structure Assay: Population Profile and Correlation with Semen Parameters and Sperm Function Test

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SCSA has been used to estimate the degree of sperm DNA fragmentation and has been linked to poor embryology and reduced pregnancy rates. Preliminary population analysis indicated that the SCSA could not be predicted from any current semen analysis parameter or a sperm function test (ARIC). In this study, only 5% of samples exceeded the DFI threshold value of 30%. The value of SCSA therefore needs to be viewed as a novel and infrequent factor in male infertility that may not be predicted from current standard tests that control for fertilisation failure. This independence argues that tests for DNA fragmentation of which SCSA is one should be included as a separate procedure in the pantheon of tests used in fertility investigations.

Aim: To Review the relationship between the Sperm Chromatin Structure Assay (SCSA) and population parameters, standard semen parameters and the Acrosome Reaction Ionophore Challenge (ARIC).

Method: The SCSA was performed according to the method described by Everson (2000) using samples diluted to 5 million/ml and frozen in LN₂. The samples were thawed immediately before the assay. All assessments were performed on a FACS Calibur Flow Cytometer. All patients attending for 3 months were assessed as part of the couple's initial investigations and the data was reported as %DFI (DNA Fragmentation Index).

Results: 103 consecutive samples were assessed. The mean DFI was 12.9 ± 9.79 with a kurtosis of 7.5 and a skewness of 2.3. The Median value was 10.3 and the mode was 9.4. The range fell between 2.1 and 63.4 and 5 samples fell outside the generally agreed threshold of 30% (4.9% of samples). There was no relationship between either the DFI value and semen parameters [concentration, motility and morphology (Strict)] or the ARIC result.

Conclusions: The population statistics indicate that the DFI assessment formed a tight assay around the mode falling between 3% and 21%. Only 5% of samples exceeded the threshold of 30% and warranted repeat assessment. There was good agreement between repeat samples confirming the reproducibility of the assay. Since there was no relationship between any semen parameters (in this study group) or sperm function test that investigate the fertilisation potential of spermatozoa, it is concluded that Sperm DNA Fragmentation is an infrequent event that has no correlation to the fertilisation process. SCSA therefore may influence other outcomes eg as has been reported for embryo quality or pregnancy, in 1 couple in 20. The value of the SCSA needs to be viewed in this context.

Validity of the IVF league tables: is it all about location, location, location?

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Background: It has been suggested that comparing the live birth rate of IVF cycles started in couples having their first IVF cycle and in whom the female is under 35 years of age and has a normal FSH level could improve the validity of the league table of IVF clinics published annually by the HFEA. We sought to test the applicability of this hypothesis through comparing the outcome of this "standard group" of patients after separating them into two groups based on their residence within the greater London area in an attempt to reflect the diversity of patients' characteristics seeking fertility treatment in the different IVF clinics.

Methods: Analysis of all IVF/ICSI cycles performed between April 2001 and April 2003 on patients who fulfilled the study inclusion criteria and used the mid-luteal down regulation protocol for ovarian stimulation was conducted. The study population were divided into 2 groups according to the referring PCT; group A (n = 90) were referred from Lambeth, Southwark and Lewisham (LSL) PCT and group B (n = 134) were referred from Brent and Harrow (BH) PCT.

Results: There was no significant difference between the two groups with regard to demographic data and IVF cycle characteristics, including female age, basal FSH and estradiol levels, dose of FSH used for ovarian stimulation, duration of ovarian stimulation, proportion of cycles reaching embryo transfer and mean number of oocytes retrieved and fertilised normally, mean number of embryos replaced per cycle and proportion of cycles achieving embryo cryopreservation. However, the two groups differed significantly in their ethnicity; group A (LSL) were predominantly of Caucasian (47%) and black African-Caribbean (37%) ethnicity, while group B (BH) patients were largely of Asian (52%) or Caucasian (30%) origin. In addition, the type of infertility (primary or secondary), presence of uterine fibroids, smoking and alcohol consumption habits were significantly different between the 2 groups. Moreover, the two groups differed in their causes of infertility; couples in group A (LSL) were significantly more likely to present with a tubal cause of infertility (RR = 2.4, 95% CI 1.6–3.5, P < 0.01), while couples in group B (BH) were more likely to have anovulatory infertility (RR = 3.5, 95% CI 1.4–8.7, P < 0.01). The implantation, clinical pregnancy and live birth rates per cycle started were significantly different between the two groups; group A had a significantly lower implantation rate (OR = 0.42, 95% CI 0.19–0.94, P = 0.003), clinical pregnancy rate (OR = 0.41, 95% CI 0.2–0.84, P = 0.006), and live birth rate (OR = 0.45, 95% CI 0.21–0.95, P = 0.02) compared with group B. After adjusting for confounding variables using multivariate logistic regression analysis, group A patients (LSL) remained significantly less likely to achieve a live birth after their first IVF cycle compared with group B (BH) patients (adjusted OR = 0.44, 95% CI 0.19–0.97, P = 0.04).

Conclusion: The study confirms the impact of the non-IVF related patient characteristics on treatment outcome and emphasises the poor validity of comparing IVF clinics' success rates based on the sparse data published by national IVF registries.