Outcome studies of effects of interventions in early life

1. Outcome of children born to mothers with renal disease in pregnancy- PORD study.

2. Outcome of children born after preimplantation genetic diagnosis/screening- PGD/S study.

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It is unwise to be too sure of one's own wisdom. It is healthy to be reminded that the strongest might weaken and the wisest might err: Mahatma Gandhi

This thesis is dedicated to,

my little girl Uditi,

my husband Ritwik,

my mother Gouri Datta,

and all my teachers without whose love, help and guidance I wouldn't be here.

Declaration

I, Indrani Banerjee, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

A research assistant supervised by the Departmental psychologist Xenya Kantaris scored the psychological questionnaires.

Dr. Mark Shevlin, who was the statistical advisor of the project, carried out the statistical analysis. The results were interpreted by me when work was presented in this thesis.

My supervisor, Dr. A. Sutcliffe guided me through the process of study designing, protocol writing, data presentation and interpretation. I independently carried out all the tasks with appropriate corrections made by Dr. Sutcliffe.

Signature: ……

Abstract

Objectives

- To assess two cohorts of children exposed to adverse/atypical in utero environments. The first group was born to mothers with chronic renal disease in pregnancy (PORD). The second group was born after pre implantation genetic diagnosis/screening (PGD/PGS). The aim was to describe the health of these two groups of children in terms of physical/neurodevelopmental and behavioural well being (in relation to a matched control group of children born to healthy mothers).
- To assess any impact from having a child whilst undergoing treatment for chronic renal disease on the psychological health of the mother and/or maternal child bonding/relationships.
- To consider specific factors in the management of maternal renal disease which may have deleterious effects on the child's outcome (e.g. fetal drug exposure)
- To assess any impact of the difficulties of having a child after PGD/PGS, which can often be stressful for couples, on the psychological health of the parents / or parent-child bonding/ relationships.

Methods

- Two population based case control studies of 176 children (and their families): One of 24 children born to mothers with chronic renal disease in pregnancy and one of 49 children born after preimplantation genetic diagnosis/screening.Controls were 37 children born to well mothers without renal disease and 66 children born after natural conception.

Outcome measures included:

- A full physical examination of the child, which included assessment of growth and general health.
- An assessment of development using Griffiths Mental Development Scales.
- Questionnaire-based to assessment of parent child relationships

Results

- Study and control children in both cohort studies were comparable for growth parameters and neurodevelopmental scores as assessed by the Griffiths Scales of Mental Development.
- The children showed no between group differences in the temperamental characteristics perceived by mothers. There was no evidence of more stress amongst study group mothers or evidence of impaired bonding between mother and child in comparison with controls.
- The PGD group had a significantly higher score on the warmth-affection sub-scale, and significantly lower score on the aggression-hostility and rejection sub-scales than the control group.
- The PORD group demonstrated more externalising behavioural difficulties. although the study reported that families (with renal disease) were more likely to be from lower socio-economic backgrounds. Significantly fewer vaginal deliveries were reported for mothers with renal disease and their infants were more likely to experience neonatal morbidity.

Summary of findings from the PORD and PGD/S studies.

PORD study

The results of this study were generally reassuring for the families where the mother have chronic renal disease and have had children.

Study and control children were comparable for growth parameters and neurodevelopmental scores as assessed by the Griffiths scales of mental development. Numbers were small. However the data does provide reassurance to a group of mothers with a variety of renal disease that there was no effect related to maternal disease or medications used on growth and development of the children.

The study highlights significant differences in externalising behaviour (e.g. rule breaking and aggressive behaviour) between the study and the control groups. The numbers involved were small and further studies would be needed to establish this. The result might relate to the comparative social disadvantage (as assessed by the

social class classification) seen in a higher proportion of PORD mothers than control mothers.

There was no difference in the temperamental characteristics perceived by mothers in study and control groups. There was no evidence of more stress amongst mothers with renal disease or evidence of impaired bonding between mother and child in comparison with controls.

The number of cases in the study was insignificant to provide strong evidence about the relation of severity of renal failure and outcome of the children. This was further compounded by difficulties in gathering maternal data from case notes.

Even though there were only eight mothers post transplant and the study provided some preliminary data to suggest that the well-being of these children were comparable with that of children born to well mothers. However, further larger studies are needed in the future.

PGD study

This study is the first detailed study of children born after PGD world wide who were over a year of age and provides provisional reassurance that these children are healthy in comparison to naturally conceived children.

Growth parameters and neurodevelopmental scores were comparable in the study and the control group, providing reassuring information for couples who have undergone the procedure and also future couples who will be undergoing the procedure. The children studied did not show any temperamental, behavioural or emotional difficulties.

The PGD group had significantly higher scores on the warmth-affection sub-scale, and significantly lower scores on the aggression-hostility and rejection sub-scales than the control group. There was also no indication of increased levels of stress related to parenting.

Conclusion

- The studies in this thesis are reassuring in terms of physical and neurodevelopmental health of children born to mothers after chronic renal disease in

pregnancy and of children conceived following pre implantation genetic diagnosis/screening and their family relationships.

Table of contents Page

List of tables

List of figures

CHAPTER 2

CHAPTER 3

CHAPTER 4

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List of Abbreviations

ART	Assisted reproductive therapies
CBCL	Child behaviour checklist
CP	Cerebral Palsy
ESHRE	European Society of Human Reproduction and Embryology
ESRD	End stage renal disease
FISH	Fluorescent in situ hybridization
GFR	Glomerular filtration rate
GHQ	General health questionnaire
GMDS	Griffiths mental development scales
HD	Haemodialysis
HFEA	Human fertilisation and Embryology Authority
HLA typing	Human leucocyte antigen typing
ICSI	Intracytoplasmic sperm injection
IDDM	Insulin dependent diabetes mellitus
IUGR	Intrauterine growth retardation
IVF	In itro fertilisation
LBW	Low birth weight
mg/dl	milligram per deci litre
mmol	milli mol
NICU	Neonatal unit
NNU	Neonatal intensive care unit
PARQ	Parental acceptance and rejection questionnaire
PCR	Polymerase chain reaction
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
PSI	Parenting stress index
RPF	Renal plasma flow
SD	Standard deviation
TTN	Transient tachypnoea of new born

Chapter 1

1. Foreword

Normal development may be disrupted by early adverse environmental influences; individuals who survive may have to cope with potentially damaging consequences. Additionally, the effects of environmental challenges in early life and the responses required to deal with them may have long-term effects into adulthood (Gluckman *et al.*. 2005d). Barker *et al.* have clearly shown that the quality of fetal life can have profound effects on future well being of offspring (Barker *et al.*. 2002;Barker 2004;Poulter 2001a).

The early phase of life in many species is characterized by the presence of responses to environmental stimuli. This developmental plasticity is important in generating a range of phenotypes suitable for different environments thus promoting the perpetuation of the genotype (Bateson 2001;Bateson *et al.*. 2004). The human fetus and infant are no different from most other mammalian species in this respect and can respond to nutritional imbalance and other adverse influences by changing their developmental and growth trajectories (Gluckman & Hanson 2004a). The processes involved may include induction of attributes that adapt the individual for the type of environment in which he or she is likely to live later in life. Development of an individual obviously depends on both his or her genes and the environment, but the precise character of the interplay between the developing individual and its environment is critical. At each phase of development the organism may be sensitive to particular environmental cues with the effects of its responses impacting on subsequent stages of development (Gluckman *et al.*. 2005c). Developmentally disruptive events in response to environmental stimuli can irreversibly interfere with embryonic development and, depending on their nature, may have deleterious effects either in utero and/or after birth. Generally, such events act by interfering with a developmental process during periods of vulnerability, such that structural deficits may emerge, although more subtle effects will be harder to detect. The stimulus may

be a drug, ionizing radiation, a major environmental shift such as hyperthermia or hypoxia, disease, or a gross nutritional disruption (e.g. folate deficiency leads to neural tube defects) in humans and other mammals. In mammals, environmental influences that have long-term consequences can operate even on the pre-ovulatory oocyte and pre-implantation embryo (Gluckman *et al.*. 2005b). This increases the concerns about the possible long term effects of human in vitro fertilisation (Jackson *et al.*. 2004). Several explanations have been offered for the long-term effects on the offspring of an altered maternal environment (Gluckman & Hanson 2004b;Hales & Barker 2001;Hattersley & Tooke 1999;Singhal *et al.*. 2003). Growth as measured at birth is a proxy for in-utero growth and prenatal experience.

In the studies described in this thesis there are two distinct exposures of interest. In the larger study the health of babies/young children born after Preimplantation Genetic Diagnosis was investigated in a cross sectional study in considerable detail. Using the same protocol another smaller study was conducted looking at the health of children aged eight or under whose mothers had had chronic renal disease in pregnancy.

Chapter 2

2. Introduction

2.1 The Paediatric outcome in maternal renal disease in pregnancy study (PORD study)

2.1.1 Introduction to the PORD study

The editorial comment "children of women with renal disease used to be born dangerously or not at all — not at all if their doctors had their way," (1975), reflects an early view of the effect of kidney disease on pregnancy. In 1971 Confortini *et al.* (Confortini *et al.*. 1971) reported the first conception and successful delivery in a woman on maintanance haemodialysis (HD). The first successful pregnancies following maternal renal transplantation were reported in 1963 (MURRAY *et al.*. 1963) .The donor and recipient were identical twin sisters. The child born turned 48 years old on March 10, 2006 (McKay & Josephson 2006f). Subsequent progress in management of end stage renal disease, ultimately by means of renal transplantation, has resulted in a lot more women living their lives into child bearing years. Such women were often discouraged from having a family due to concerns about posssible adverse effects on renal grafts and on the potential offspring (Willis *et al.*. 2000f).

2.1.2 Pregnancy in women with moderate or severe renal insufficiency

The outcome and the consequences of pregnancy in women with impaired renal function are still debated. Fertility and ability to sustain an uncomplicated pregnancy generally relate to the degree of functional impairment and the presence or absence of hypertension (Lindheimer, Grunfeld, & Davison 2007).

In an update of renal disorders in pregnancy, John M Davison divides women arbitrarily into three categories (Davison 2001c): Refer to table 2.1:

Table 2.1 Categories of chronic renal disease

Serum creatinine (Scr)	Renal Function
≤125 μmol/l or 1.4 mg/dl (Gr 1)	Preserved or only mild impairment of renal function
125-250 μmol/l or 1.4-2.8 mg/dl(some use 220 μmol/l or 2.5 mg/dl as the cut-off) (Gr 2)	Moderate renal insufficiency,
>250 μmol/l or 2.8 mg/dl (Gr 3)	Severe renal insufficiency

He reported that women in Group 1 always have adequate gestational increments in Renal Plasma Flow (RPF) and Glomerular Filtration rate (GFR) which are markers of increased renal haemodynamics in pregnancy. In this group the obstetric outcome and the long-term renal prognosis for mothers were good. In groups 2 and 3 the increase of renal haemodynamics in pregnancy was less predictable and the long-term renal prognosis was uncertain. Many mothers, however, suffer significant renal function loss during pregnancy, with an accelerated decline post-delivery. However in groups 1 & 2 obstetric success is more likely because of better fetal surveillance and advances in neonatal intensive care facilities (Davison 2001h). He highlighted hypertension, which if significant and poorly controlled still continues to be an indicator of bad prognosis for mother and baby (Davison 2001b)

Davison's update highlighted a single centre study by Bar *et al.* (Bar *et al.*. 2000b). This study from Israel analysed types and frequencies of short and long-term (2 years after delivery) maternal and neonatal complications in three patient subgroups. The patient groups were 38 patient with primary renal disease (46 pregnancies), 24 IDDM patients with diabetic nephropathy (24 pregnancies) and 24 patients with functional renal allograft (42 pregnancies). Most of them had mild renal impairment. Successful obstetric outcome was defined as 'a live healthy infant without severe handicap 2 years after delivery'. Success was reported in 98% of patients with primary renal disease, 96% of IDDM patients with diabetic nephropathy and 89% of patients with functional renal allograft. Factors found to be predictive of successful outcome were absence of pre existing hypertension in all groups, low preconception serum uric acid level in primary renal disease patients, a long interval from transplantation to conception and use of low dose prednisone in renal transplant patients. They

concluded that worse pregnancy outcome occurred in women with moderate or severe renal insufficiency. The findings from the study are summarised in the tables 2.2, 2.3 and 2.4.

Table 2.2: Short Term pregnancy outcome in the study group.

	Group			
	Primary Renal Disease	Diabetic Nephropathy	Renal Transplantation	p value
Superimposed pre eclampsia	10(22%)	11(46%)	7(17%)	0.05
Preterm delivery (%)	10(22%)	4(17%)	26(62%)	0.01
IUGR(%)	6(13%)	5(21%)	14(33%)	<0.05
Caesarean delivery(%)	11(24%)	15(62.5%)	15(36%)	0.001
Hospitalisation in NICU(%)	2(4.4%)	1(4.2%)	14(33%)	0.02
Stillbirths (%)	0	1(4.2%)	2(7%)	N.S

Bar, Ben-Rafael, Padoa *et al.*, 2000

Table 2.3: Long term (two years after delivery) outcome in the study groups.

	Group			
	Primary Renal Disease	Diabetic Nephropathy	Renal Transplantation	p value
Significant increase in serum creatinine (>1mg per dl) (%)	2(5%)	0	4 (15%)	NS
End-stage renal disease	0	0	3(11%)	NS
Severe handicap or late infant death (%)	1(2.2%)	2(8.4%)	2(7%)	NS

Bar, Ben-Rafael, Padoa *et al.*, 2000

Table 2.4: Characteristic and short- and long term pregnancy outcome in patients with moderate or severe renal insufficiency in the study groups.

	Group	
	Primary Renal Disease	Renal Transplantation
No of patients	4	9
No. of pregnancies	5	14
Mean age (years) (range)	28.5 ± 2.1 (25-30)	29.4 ± 3.6 (22-35)
No. with pre-existing hypertension	1(25%)	7(78%)
Superimposed pre-eclampsia	1(25%)	2(22%)
Preterm delivery	2(50%)	8(89%)
Still births (%)	0	0
Significant increase in Serum creatinine (>mg/dl) (%)	1(25%)	4(44%)
End-stage renal disease	0	3(33%)
Severe handicap or late infant death	0	1(11%)

Bar, Ben-Rafael, Padoa *et al.*, 2000

The children's well being (absence of severe handicap in the form of cerebral palsy, blindness, deafness or late death) were determined in a clinic in the children's hospital (no details available) affiliated with the study centre. No details of the assessments or data regarding the health of the children were described in the paper (Bar *et al.*. 2000a).

Jungers and colleagues report (1997) from France was in accord with the wide consensus that pregnancy in women with renal disease and normal or near normal renal function is most often successful and usually does not result in deterioration of the maternal renal condition (Jungers *et al.*. 1997c). This was supported by several other studies in the past (Abe *et al.*. 1985;Barcelo *et al.*. 1986;Jungers *et al.*. 1986;Jungers *et al.*. 1987;Jungers *et al.*. 1991;Katz *et al.*. 1980;Packham *et al.*. 1989;Surian *et al.*. 1984). Jungers *et al.* reported a similar outcome in 1995 in a large series of women with primary glomerulonephritis whose serum creatinine was below 0.11 mmol/l at the start of pregnancy. In contrast, outcome of pregnancy in terms of both fetal and maternal outcome was much worse in women with primary renal disease whose renal function was significantly compromised at conception. Fetal loss rate (not taking into account first trimester abortions) as high as 16-33% was reported by Hou *et al.* 1985, (Becker *et al.*. 1986;Imbasciati *et al.*. 1986). There were high proportions of premature deliveries and low birth weight infants. In addition the authors also reported that pregnancy markedly accelerated the underlying maternal renal disease. Jungers' (Jungers *et al.*. 1997b)retrospective analysis of 43 pregnancies

in women with moderate to severe chronic renal failure during the 20-year period from 1975-1994 showed the following: Tables 2.5 and 2.6

Table 2.5: Maternal characteristics and fetal outcome with respect to serum creatinine level at start of pregnancy (after Jungers *et al.* 1997)

Scr (mmol/l)	Group I (0.11-0.20)	Group II (>0.20)	p value
No. of pregnancies	28* (30 fetuses)	15	
Status at conception			
Age (years)	25.6± 0.6	28.7± 0.8	0.003
MAP (mmHg)	98± 2	103±3	0.16
Haemoglobin (g/dl)	12.3± 0.2	10.9± 0.3	<0.001
Scr (mmol/l)	0.143± 0.004	0.254± 0.019	<0.001
Fetal outcome			
1st trimester abortion	1 (3%)	4 (27%)	0.04
Stillbirth	5 (17%)	3 (20%)	NS
Live born infants	24/30* (80%)	8/15 (53%)	0.02
preterm delivery	14/24* (58%)	4/8 (50%)	NS
birth weight	2437 ± 128	2246 ± 156	NS
Corrected live birth rate**	24/29 (83%)	8/11 (73%)	NS

* Including 2 sets of twin
** Not considering first-trimester abortions

Table 2.6: Fetal outcome with respect to the period of observation (after Jungers *et al.* 1997)

Observation period	Period I (1975-1984)	Period II (1985-1994)	p value
No. of pregnancies	20	23 (25fetuses)*	
Status at conception			
Age (years)	26.3 ± 0.8	27± 0.7	NS
MAP (mmHg)	103±2	97± 2	0.03
Scr>0.20 mmol/l	6/20 (30%)	9/23 (39%)	NS
Haemoglobin (g/dl)	11.7 ± 0.3	12 ± 0.3	NS
Fetal outcome			
1st trimester abortion	3 (15%)	2 (8%)	NS
Stillbirth	6 (30%)	2 (8%)	0.11
Live born infants	11/20 (55%)	21/25* (84%)	0.048
preterm delivery	4/11 936%)	14/21* (67%)	0.14
birth weight	2648 ± 138	2211± 174	0.08
Corrected live birth rate**	11/17 (65%)	21/23 (91%)	0.053

* Including 2 sets of twin
** Not considering first-trimester abortions

The study concluded that fetal outcome in patients with impaired renal function had improved in recent times due to improvements of obstetric and neonatal care, improved blood pressure control and multidisciplinary care of such mothers by teams of nephrologists and obstetricians. However, the risk of fetal loss and accelerated

decline of maternal renal disease still remained especially with a creatinine clearance at conception is lower than 25-30ml/min/1.73m^2.

Jones and Hayslett (Jones & Hayslett 1996a) performed a study to ascertain maternal complications, immediate and long term effects of the pregnancy on underlying disease and outcome of pregnancy in women with moderate or severe renal disease at the beginning of pregnancy. They highlighted that the fetal survival was moderately (overall fetal survival rate was 93%) reduced and more than half the pregnancies were associated with obstetric complications. Growth retardation, prematurity and maternal complications were identified as factors which were most often responsible for caesarean section and admission to a neonatal unit. The table 2.7 below summarises the findings from the study:

Table 2.7: Outcomes of 82 pregnancies and neonatal complications

Variable	Value
Preterm delivery (<37 wks) – no. (%)	48(59)
Induction of labour – no. (%)	8(10)
Delivery by Caesarean section – (%)	48(59)
Indication	
Fetal distress	4
Intrauterine growth retardation	11
Macrosomia	1
Dystocia	2
Breech position	2
Maternal renal deterioration	5
Maternal hypertension and pre eclampsia	11
None reported	12
Mean (±SD) birth weight – g*	2239 ± 839
Small for gestational age (<10th percentile) - no. (%)	28 (37)
Death – no. (%)	6(7)
Stillbirth – rate per 1000 births	49
Neonatal death – rate per 1000 births	24
Admission to neonatal intensive care unit - no. (% of live born infants)	28(37)
Indication	
Prematurity, IUGR or both	25
Sepsis	1
Unknown	2

* Data on birth weight were available for only 76 of the 82 pregnancies

The study included a follow up of nearly all mothers for 12 months after delivery and showed the following:

- Pregnancy related loss of renal function (a loss occurring during pregnancy or within six weeks after delivery) in nearly half of all cases.
- 23% of these (10% of total series) women were rapidly progressing to end-stage renal failure within six months of delivery.
- Excluding the women with accelerated progression to end stage renal failure, the postpartum serum creatinine concentration was comparable to that of women with stable renal function during pregnancy. This was a generally positive message for women contemplating pregnancy.

However, the effect of renal disease on the growth and development of the surviving infants was not evaluated any further than the 12 months.

While the studies above provided useful guidelines for counselling women with pre-existing renal insufficiency about their prospects of a successful pregnancy and the effect of pregnancy on their underlying disease, there was no long term follow up data regarding the health and development of children born to these mothers.

2.1.3 Pregnancy in women on dialysis

Pregnancy used to be a rare occurence in women on dialysis. Several factors contributed: hyperprolactinemia causes anovulation often manifest in these women as amenorrhoea, oligomenorrhoea or polymenorrhea (Redrow *et al.*. 1988). In women who do ovulate and menstruate regularly, abnormal endometrial maturation may prevent implantation. Furthermore, an uraemic environment is detrimental to the developing embryo (Ginsburg & Owen 1993;Gomez *et al.*. 1980). In 1971 Confortini *et al.* (Confortini *et al.* 1971) reported the first successful pregnancy in a woman on chronic hemodialysis (HD). Although still uncommon, the incidence of pregnancy in women on dialysis may be increasing. In 1980 the European Dialysis and Transplant Association (EDTA) reported a pregnancy incidence of 0.9% (1980b). Recent publications report pregnancy in 1–7% in women on chronic dialysis (Bagon *et al.*. 1998b;Chao *et al.*. 2002b;Hou 1994a;Okundaye *et al.* 1998a;Rizzoni *et al.*.

1992;Souqiyyeh *et al.*. 1992;Toma *et al.*. 1999d). The true frequency of conception is unknown because many pregnancies in dialysed patients probably end in early spontaneous abortion, and there is also a high therapeutic abortion rate (Davison 2001i).

Nowadays, pregnancy in contemporary women on dialysis is more likely to be successful, with 30–50% of pregnancies resulting in delivery of a surviving infant (Holley & Reddy 2003g). Premature delivery, intrauterine growth retardation, polyhydramnios, and maternal hypertension remain common complications. Newborn weight and survival appear to be influenced by dialysis dose. Thus increasing total weekly dialysis time during pregnancy via increased frequency of treatments leads to a longer gestation, higher infant birth weight, and more viable pregnancies (Bagon *et al.*. 1998a;Okundaye, Abrinko, & Hou 1998b;Rizzoni *et al.* 1992)

The following tables summarise recent reports of pregnancy in women on chronic dialysis.

Table 2.8: Incidence and outcome of pregnancy in women on chronic HD (Modified from Holley J L. & Reddy SS, 2003 (Holley & Reddy 2003e))

Reference	Year	No. of pregnancies/no. of women	Incidence of pregnancy	Surviving infants	Neonatal deaths	Spontaneous abortion
Souquiyyeh MZ et al.	1992	27/380	7%	30%	—	—
Hou SH	1994	58/1281	1.5%	37%	5%	44%
Okundaye I et al.	1998	184/6230	2.2%	40%	3%	46%
Bagon JA et al.	1998	15/1472	1%	50%	13%	—
Toma H et al..	1999[a]	172/5000	3.4%	49%	5%	12%
Chao A-S et al.	2002	18	—	50%	17%	—

[a] Detailed information on 74 pregnancies;
—, not reported.

Table 2.9: Duration and complications of pregnancy in women on chronic HD
(Modified from Holley J L. & Reddy SS, 2003 (Holley & Reddy 2003d))

Reference	No of pregnancies	Mean pregnancy (weeks)	Mean newborn weight	Incidence of polyhydramnios	Incidence of maternal hypertension	Caesarean section deliveries
Okundaye I et al.	184	32.4 ± 4.6	—	—	79%	—
Bagon JA et al.	15	—	1164 (700–1900)	62%	—	8 (53%)
Toma H et al..	68	31.9	1543.5 ± 671.9 (530–2856)	33%	42%	—
Chao A-S et al.	18	32	1542 (512–1660)	46%	72%	6 (46%)

—, not reported.

Table 2.10: Complications and Outcomes of Pregnancy in Chronic haemodialysis Patients (Modified from Reddy SS and HolleyJL, 2007) (Reddy & Holley 2007c)

Refences Author	Year	No. of treated pregnancies	Mean Pregnancy (weeks)	Mean newborn Birth weight	Mternal hypertension (%)	Incidence polyhydramnios	% Caesearean Section	Fetal/neonatal Deaths (%)	Surviving infants No. (%)
Bagon	1998	15	30.6	1164g (700-1600g)	62	—	53	5(33)	10 (67)
Romao	1988	14	32.3±2.6 (27-36)	1400 ± 579 (720-2650g)	50	36	(67) 8 out of 12 known	3 (21)	11 (79)
Toma	1999	54	31.9±	1544 ± 672 (530-2856)	35	44	—	18(33)	36 (67)
Chao	2002	13	32	1542 (512-1660)	72	46	46	4 (31)	9 (69)
Kazancioglu	2002	3	31	1250	67	67	67	1(3)	2(67)
Eroglu	2004	7	32 (26-36)	1400 (420-2640)	—	29	57	1(14)	6 (86)
Hasse	2005	5	33	1765±554	40	40	80	0(0)	5(100)

— not reported

A report from a series of three successful pregnancies in women with chronic kidney disease (Brem *et al.*. 1988a), two of whom were treated with haemodialysis, stated that all infants were premature and had low birth weights. The infants were azotaemic immediately after birth, which rapidly resolved, as the fetal renal function was good. An osmotic diuresis caused 4-8% drop in weight in the first 48 hours of life. This led to hypokalaemia and volume contraction in the infants. There were no major growth or developmental abnormalities in these children in the first year of life, but there was no reported long-term follow up (Brem *et al.*. 1988b).

While most pregnant dialysis patients have been on haemodialysis HD, case reports and surveys also detail successful pregnancies in women on chronic peritoneal dialysis (PD) (Hou 2001;Hou 1994c;Jakobi *et al.*. 1992;Okundaye, Abrinko, & Hou 1998d) The incidence of pregnancy in women on PD is two to three times lower than that of women on HD.

As debate continues about the advisability or not of pregnancy in dialysis patients, more and more women are choosing to take the chance because childbearing and parenthood are important rehabilitation goals (Davison 2001a). There are no explicit studies that describe the health of infants or children born to mothers having on-going haemodialysis or peritoneal dialysis. The above papers gave little or no details of child outcome.

2.1.4 Pregnancy in women with renal transplant

When human organ transplantation was first developing, the physicians worried about the potential teratogenicity of the immunosuppressive drugs used in these mothers and pregnancy was ill advised (McKay & Josephson 2006c). Despite these concerns, approximately 14,000 births worldwide have been reported among women with transplanted organs, the most common being renal transplant. With ongoing improvements in surgical, immunological and medical management of transplant patients many more pregnancies are now occurring (McKay & Josephson 2006d). Pregnancy is now an expected benefit afforded to women by organ transplantation.

Fertility in women with chronic renal failure is low due to a combination of factors like loss of libido, ovarian dysfunction, anovulatory vaginal bleeding, amenorrhea and high prolactin levels (Zingraff *et al.*. 1982). After renal

transplantation there is rapid restoration of renal, endocrine and sexual function (Davison & Bailey 2003f).

Information about pregnancy in recipients of solid-organ transplant came from voluntary registries, case reports and retrospective centre studies. Three registries offered data about pregnancy outcomes: the National Transplantation Pregnancy Registry in the United States, the European Dialysis and Transplant Association Registry and the United Kingdom Transplant Pregnancy Registry (McKay & Josephson 2006e). Most Transplantation centers have advised that conception was safe after the second post transplantation year, assuming that the graft functioned well. Criteria as set out in the European Best Practice Guidelines IV which women with renal transplant need to meet were: stable serum creatinine level of 1.5mg/dl (133μmol/l) or less and a rate of urinary protein excretion less than 500mg per day (2002b).

In an update of 'Renal disorders in pregnancy', John M Davison (Davison 2001j) reported that outcome of pregnancy is directly related to the following:

a) Better allograft function
b) Low steroid dosage before conception
c) Longer time interval between transplant and conception
d) Transplanted kidney comes from a living donor.

However, a recent report from the UK transplant pregnant registry stated that there was no statistically significant difference between the proportion of successful pregnancies in cadaveric and live donor transplant recipients (Sibanda *et al.*. 2007g).

About 1 in 50 women of child bearing age with a functioning renal transplant becomes pregnant (Lindheimer, Grunfeld, & Davison 2007). Women with renal allografts who conceived had a high incidence of maternal and fetal complications. About 25% of pregnancies miscarried or were terminated; of the remainder 95% or more were successful (Davison & Bailey 2003e). John Davison and David Bailey summarised from their report in August 2003 the following maternal and fetal complications: refer to tables 2.11 and 2.12 (Davison & Bailey 2003d).

Table 2.11: Pregnancy outcomes in renal allograft recipients (modified from Davison and Bailey 2003)

First Trimester Lossess (n = 81)	22.5%
Miscarriage	13%
Termination	9%
Ectopic pregnancy	0.5%
Pregnancies beyond 1st trimester (n = 296)	
Live births	97%
Stillbirths	2%
Neonatal deaths	1%

Three hundred and seventy–seven pregnancies in 261 renal allograft recipients.
(US National Transplantation Register)

Table 2.12: Maternal risks of renal transplantation and immunosuppression (modified from Davison and Bailey 2003)

Increased risk of ectopic pregnancy
Hypertension and pre-eclampsia
Bacterial and viral infections consequent to immunosuppression
Toxic effects of immunosuppressant drugs
Pelvic osteodystrophy
Increased likelihood of operative delivery

Table 2.13: Fetal risks of renal transplantation and immunosuppression (modified from Davison and Bailey 2003)

Intrauterine growth restriction, hypoxia and death
Preterm delivery
Preterm labour
Preterm premature rupture of membranes
Preterm delivery because of maternal complications
Toxic effects of immunosuppressnat drugs
Depressed haemopoiesis
Immune deficiency
Chromosome aberrations in leucocytes
Adrenocortical insufficiency
Infection
Cytomegalovirus
Hepatitis B
Bacterial sepsis

Davison (Davison 2001f) also stated that there were increased rates of late pregnancy maternal hypertension (30%), preterm delivery (45-66%) and fetal growth restriction (upto 40%). Some of these problems may be on the rise with the use of cyclosporin and more recent immunosuppressive agents.Refer to table 2.14:

Table 2.14: A total of 424 livebirth outcomes in female renal transplant recipients reported to the National Transplantation Pregnancy Register
Modified from Armenti *et al.* (Armenti *et al.* Graft2000;3:59-63)

		CsA	Neoral	Tacro
Live births(n)		350	57	17
Gestational age (mean) (weeks)	35.9	35.8	33	
Birthweight(mean) (g)	2485	2449	2151	
Premature(<37weeks)(%)	52	51	63	
Low birthweight(<2500 g) (%)	46	54	63	
Caesarean section (%)		51	48	44
Newborn complications (%)		40	49	53
Neonatal deaths (within 30 days of birth) (%)	1	0	6	

CsA, Sandimmune brand cyclosporin; Neoral,Neoral brand cyclosporin; Tacro,tacrolimus, FK506 or Prograf.

2.1.5 Complications reported in pregnancies post renal transplant

A. Neonatal complications

A.1 Neonatal morbidity

Neonatal morbidity is mainly a consequence of prematurity (50% of babies) and growth restriction (54% babies were low birth weight i.e. <2500gm) (Sibanda *et al.*. 2007f) which may be co-existing. There is an association between low birth weight, birth within two years of transplantation (Cunningham *et al.*. 1983) and cyclosporin therapy (Armenti *et al.*. 2002;Armenti, Moritz, & Davison 1998b).

A.2 Adrenal insufficiency

Penn *et al.* reported evidence of adrenocortical insufficiency and lymphopenia in two infants born to mothers with renal transplant (Penn *et al.*. 1971b). Both the infants had hyponatraemia, hyperkalemia and lymphopenia. One infant became hypotensive and lethargic and required treatment with corticosteroids. The possibility of thymic hypoplasia was raised. Both the infants made full recovery without immediate residual problems. There was also a report of twin pregnancy where both infants had lymphoid hypoplasia and hypoplasia of adrenal cortex at post mortem (Lower *et al.*. 1971).

A.3 Neonatal infections

Neonatal bacterial infection was infrequent and when encountered it responded appropriately to therapy. Pregnant allograft recipients are predisposed to viral infections because of immunosuppression. Heaptitis B and cytomegalovirus are particularly associated with renal transplant mothers and pose a risk both for the fetus and neonate. However the rate of these infections are not reported in the literature. Evans in 1975 (Evans, McCollum, & Valdimarsson 1975) reported congenital CMV infection after maternal renal transplantation. There is also one report of an infant developing asymptomatic antigenemia in a mother who was HB_sAg positive (Salant *et al.*. 1976).

B. Effects of maternal transplant related medications

Chromosomal abnormalities were seen in neonatal white blood cell following maternal treatment with azothioprine. These may persist for up to two years. (Davison & Bailey 2003c;Leb, Weisskopf, & Kanovitz 1971). Longer lasting effects in other tissues are possible (Scott, Branch, & Holman 2002b). In female infants germ cells develop before birth and thus there is a theoretical risk that the immunosuppressants used in mothers might have a long term effect on the child's oocytes. Thus there remains at least a possibility that children born to women with transplants may suffer from malignant disease, immune compromise and abnormal reproductive performance (Davison & Bailey 2003b). Experiments in female mice given 6-mercaptopurine during pregnancy show that many of the female offspring may be sterile or if they do

become pregnant, have smaller litters and more dead fetuses than the off spring of mothers who had not received the drug (Reimers & Sluss 1978). Only prolonged follow up of the children born to transplant patients will provide an answer as to whether similar problems may occur in humans (Penn, Makowski, & Harris 1980a). Immunosuppression of the fetus is a possible adverse effect. Tests showing a depressed immune response have been described in infants born to renal tansplant recipients. Some of these abnormalities persisted at one year but without any apparent accompanying morbidity (Schena *et al.*. 2002)

Scott and colleagues (Scott, Branch, & Holman 2002a) stated that immunosuppressive agents readily crossed the placenta during the development of the fetus and its immune system. But the effects of the agents may not become evident until the offspring reaches adulthood. They reported a case of a 23-year-old daughter of a renal allograft recipient, who was exposed to azathioprine and prednisone throughout her mother's pregnancy. The daughter had stiff joint when 8 years old and developed ulcerative colitis when 16 years old. This daughter went on to have two pregnancies, one complicated by autoantibodies and fetal death and the other by systemic lupus erythematosus and preeclampsia, but with the birth of a preterm male infant. This raised the question whether autoimmune or reproductive disorders should be expected in daughters of transplant recipient. The authors recommended a cautious approach: 'parenthood may never be without risk in generations that follow transplantation but the risks are uncertain'. There is one case report associating maternal tacrolimus with neonatal cardiomyopathy (Vyas *et al.*. 1999) . Scott and colleagues have suggested that fetal exposure to immunosuppressives causes abnormalities that may be associated with later development of autoimmune disease. Autoantibodies were not found in four children exposed in utero to azathioprine or in seven children exposed to cyclosporine(Classen & Shevach 1991;Pilarski, Yacyshyn, & Lazarovits 1994).

Overall there is no definite pattern of fetal abnormalities identified with the use of maternal corticosteroids, azathioprine or cyclosporin A in either animal models or in human infants (Blowey & Warady 1998;Hou 1999c;Mason *et al.*. 1985;Reinisch *et al.*. 1978).

C. Neonatal deaths

Early fetal loss by miscarriage was reported in 11% of pregnancies in the UK Transplant Pregnancy Registry and most recent figure from the NTPR is 14% (Sibanda *et al.*. 2007e).There is no clarification regarding spontaneous and therapeutic abortions. In a follow up study by Willis *et al.,* (Willis *et al.*. 2000e) of children of renal transplant recipient mothers, reported 2 neonatal deaths representing 3.5% of the live births. Incidence figures of ectopic pregnancy, intrauterine fetal death and still-birth in the UK Transplant Pregnancy Registry are each 2% or less. However, the total number of patients in the registry were too small to compare with the general population.

2.1.6 Report of pregnancy outcome post renal transplant from Royal Free Hospital

Thompson *et al.*'s (Thompson *et al.*. 2003a) study from the renal department of the Royal Free Hospital in London looked into graft, fetal and maternal outcomes of pregnancy in their renal transplant patients and compared their results with those of the large national transplant registries. The mean time from transplantation to pregnancy was 6.5 years, with unfavourable outcome in women who conceived within 1 year of transplant. There was 41% of fetal growth restriction (FGR) and 33% of infants were small for gestational age. FGR was associated with maternal hypertension, pre-pregnancy serum creatinine $\geq$ 133μmol/l (1.5mg/dl), calcineurin inhibitors and the use of cardioselective β blockers. Pregnancy had a deleterious effect on the graft function in patients with serum creatinine > 155μmol/l (1.75mg/dl). The obstetric, maternal and fetal outcomes are as summarised in tables 2.15 and 2.16.

Table 2.15: Obstetric outcomes and maternal outcomes (modified from Thompson *et al.* 2003)

Pregnancy outcome (n=48)	
Live births	32 (68%)
Miscarriages	6 (12%)
Terminations	6 (12%)
Ectopics	2 (4%)
Stillborn	2 (4%)
Mean age at time of pregnancy	30 yrs (19-39 yrs)
Mean time between TPx and pregnancy	6.5 yrs (0.5-24 yrs)
Complications (n=27)	
Urinary tract infections in pregnancy	26%(7)
Pre-pregnacy hypertension	77% (21)
Preeclampsia	29% (8)
Delivery mode (n=32)	
Caesarean section	59% (19)
Forceps	22% (7)
Vaginal	19% (6)
Graft outcome (n=30)	
Rejection during pregnancy	0%
Graft dysfunction during pregnancy	16%
Graft dysfunction persisted (>6months) post pregnancy	20%
Graft loss <2 yrs post partum	3.3%

Table 2.16 : Neonatal outcomes (modified from Thompson *et al.* 2003)

Neonatal outcome	RFH	NTPR 2001	UKTPR
Pre-term delivery (< 37 weeks)	56.5%	52%	49%
Mean gestational age	34.9 weeks	36 weeks	---
Mean birth weight	2204 g	2493 g	---
Low birth weight (< 2500 g)	50%	45%	54%
Very low birth weight (< 1500 g)	20%		18%
Fetal growth restriction	40.7%		8%
Small for gestational age (< 10th percentile)	33%		

RFH, Royal Free Hospital; NTPR 2001; UKTPR 1999

2.1.7 What has been reported so far about children of renal tranplant recipient mothers?

Women with renal transplants have been discouraged to contemplate pregnancy due to concerns about the possible adverse effects on the children. There has been very little robust data available on the outcome of offspring of such women although there was evidence for both good and bad outcomes (Burleson *et al.*. 1983;Hou 1999b;Korsch *et al.*. 1980i;Rasmussen *et al.*. 1981).

The following table summarises the studies which have reported follow up of children born following renal transplantation:

Table 2.17 Summary of studies of children born after renal transplant

Author	Year	No of children studied	Health evaluation of children	Control group
Korsch et al.(Korsch et al.. 1980h)	1980	10 children. Age at assessment varied from10 month – 6yr 8 month	All children had normal physical examination.	No
Pahl et al.(Pahl et al.. 1993b)	1993	10 out of 26 children born. Age at assessment or duration of follow up not specified.	One child with hepatoblastoma requiring excision.	No
Ghahramani et al.(Ghahramani, Attaipour, & Ghods 1993)	1993	13 live born followed for 36 month	All children had normal physical examination.	No
Bar et al.(Bar et al.. 1996b)	1996	29 children. Age at assessment or duration of follow up not specified.	Two children reported handicapped, no specific details given.	No
Willis et al.(Willis et al.. 2000d)	2000	48 children aged between 9 month and 18 years.	General health was unremarkable. Cerebral palsy(1), claw hand (1), asthma(2), recurrent URTI(1)	Yes
Sgro et al.(Sgro et al.. 2002f)	2002	32 live born children between 3 month and 11years.	IDDM (1), asthma (2), recurrent otitis media (1)	Yes

2.1.8 Psychological aspects of pregnancy in mothers with renal transplant:

In a review of psychological issues in women with renal disease Kimmel and Patel (Kimmel & Patel 2003) stated that chronic renal disease may prevent the patient from playing roles she desires to maintain or perceives herself capable of accomplishing, or may limit her abilities compared to her premorbid status. Illness may also impair libido, sexual and reproductive function. Patients can adapt to these challenges by achieving success in several domains including maintaining employment, functioning in marital, familial and societal contexts, achieving parenthood and enjoying recreational activities. Otherwise disability, marital discord, family dysfunction and substance abuse can result. They suggest that women take the added burden of homemaking, child rearing and care taking activities within the family. Childbirth and bringing up children is often an important concern for women with renal disease. Diminished chances of successful pregnancy is often an important issue in the life of young women of reproductive age with renal disease.

Davison and Bailey (Davison & Bailey 2003a) pointed out that many women choose parenthood in an effort to re-establish a normal life in the face of a serious chronic illness. In certain situations this may bring them into conflict with their medical supervisors so that some women do not seek obstetric or medical advice until already pregnant. This often gives rise to ethical and clinical management dilemmas. There need to be more detailed studies to understand the psychology of women who pursue parenthood despite substantial risk to their own health and that of their unborn children (Stotland & Stotland 1998).

Parenthood is one of the criteria describing the quality of life after renal transplantation (Moon *et al.*. 2000b). However, even up to the1980s such women were strongly advised against planning a family. From the study of BM Korsch *et al.* from US, we see that a significant number of mothers were urged by families and physicians to terminate their pregnancies because of concerns about the possibility of graft rejection and birth defects in future off spring. Following the birth of their child, some mothers were persuaded to undergo tubal ligation to avoid future pregnancies and associated risk. It is to be noted that two women who did undergo this procedure later regretted the

decision and one sought reversal of the procedure. It is interesting to note from this report from 1980 that none of the allograft recipient parents were concerned about their life expectancy or future health as a result of being transplant patients. Most of them reported that they were enjoying parenthood, engaging in age- appropriate activities and coping appropriately with various stresses in their lives (Korsch *et al.*. 1980g). The authors had also expressed concerns about the fact that female allograft recipients often met with intensive, dogmatic and negative attitutes toward their pregnancies. Often advice was given to these mothers without exploration of their own preferences.

Another study by Penn *et al.* (Penn, Makowski, & Harris 1980b) highlighted that uncertain life expectancy of patients is a huge concern. Those desiring parenthood had to be warned that they may not live long enough to rear their children to adulthood and may leave their spouses with the problem of raising their children (Makowski 1976;Penn *et al.*. 1971a) although a single parent raising children is not always a problem.

Follow up of children of renal transplant recipient mothers by Willis *et al.* (Willis *et al.* 2000c) highlighted numerous psychological issues arising in families where the mother had undergone renal transplantation, which may influence the development of a child. This included maternal anxiety regarding her own health as well as the health of her children. The authors did not specifically assess this psychological aspect but observed that many mothers were worried about the possibility of renal disease in their children, often not discussing the issues openly with their physicians. A lot of these mothers were precluded from breast feeding because of the medications they were taking. There is increasing "pressure" in the community regarding breast feeding and this may adversely affect a women's perception of her mothering skills. Children's anxiety concerning the mothers's health (illness, potential graft failure or early death) may influence child psychological adjustment and behaviour.

Similar concerns have been expressed about women on dialysis in a recent update from Reddy and Holley (Reddy & Holley 2007b). Although the success of pregnancy is now more likely in women on dialysis, the burden on the women and her family is significant. This is not only during pregnancy but

also after delivery, when the mother must continue with dialysis and is also faced with the challenge of caring for an often premature infant.
Over the decades the medical profession has developed a more open- minded approach towards allograft recipients who wish to become pregnant. This has been helped by evidence of increasingly better outcome of pregnancies in this group and further studies looking into long term follow up of children born to mothers with renal transplant may jusitfy an even more optimistic outlook.

This overview sets the scene for the need for the PORD study. The objectives of the study were:

1. To assess a cohort of children born to mothers with chronic kidney disease and describe their health in terms of physical/neurodevelopmental and behavioural well being (in relation to a matched control group of children born to healthy mothers).
2. To assess any impact of the difficulties of having a child whilst undergoing treatment for chronic kidney disease on the psychological health for the mother / or maternal child bonding/ relationships.
3. To consider specific factors in the management of maternal chronic kidney disease which may have deleterious effects on the child's outcome (e.g. fetal drug exposure)

Hypothesis of the study was:
Children born to mothers with chronic kidney disease were expected to have the following complications in comparison to age matched children born to well mothers:

a. Reduced longitudinal growth
b. Greater occurrence of difficult temperament and emotional or behavioural problems
c. Possible effects on neurodevelopment.

These effects would be a continuum from healthy children to those with the above morbidity depending on antenatal exposures/ disease severity.
Families where children are born to mothers with chronic kidney disease would experience the following:

a. Differences in maternal bonding.
b. More stress in parent child relationship.

c. Other difficulties in parenting (related to the pressures of renal disease and treatment).

Sub hypothesis:

a. The severity of effects would be directly related to the severity (stage) of chronic renal failure.
b. Different effects would be observed in children of renal transplant recipients.

Statement of research questions for the study were:

We wished to establish the following:

a. What are the physical and neurodevelopmental outcomes for a representative cohort of these children?
b. Are there difficulties with maternal bonding and parenting skills with these mothers when compared with in relation to matched controls? (Do these mothers experience more difficulties with maternal bonding and parenting skills than their matched controls

2.2 Health outcomes of children born to parents who have had pre-implantation genetic diagnosis/screening (PGD/PGS) pilot study (PGD study)

2.2.1 Introduction to PGD study

While the problem of infertility has affected humanity for as long as there have been human beings, the technology required to fertilise a human egg in an environment outside the human body only became available in recent times.

Infertility is regarded as a health problem (WHO, Reproductive Health Strategy 2004) and affects approximately every 6th couple in Western Countries. It is however not a public health issue in every country. The number of infertile couples is on the rise: partly associated with advanced maternal age (delayed maternity) and subsequent poor quality gametes, possibly also due to related lifestyle habits and environmental factors (Soini *et al.*. 2006g).

In 1977, Dr. Robert Edwards and Dr. Patrick Steptoe pioneered IVF (in vitro fertilisation) procedure. Thanks to their work, Louise Brown, the world's first baby to be conceived in vitro, was born in the UK on July 25, 1978 amid intense controversy over the safety and morality of the procedure (Edwards, Steptoe, & Purdy 1980;Steptoe & Edwards 1978a). She gave birth on December 20, 2006 to a baby boy, who was conceived naturally. To date more than one million babies have been born world wide following assissted reproductive techniques (Sutcliffe & Ludwig 2007).

Preimplantation genetic diagnosis (PGD) is a procedure, which was developed in the early 1990's, in which embryos obtained through *in vitro* fertilization (IVF) are analyzed for a genetic disorder before the 'unaffected' embryos are implanted in the woman's uterus. As such, PGD is a very early form of prenatal diagnosis (PND) (Braude *et al.*. 2002). In Britain, as in most of Europe, Preimplantation genetic diagnosis (PGD) refers to the removal of a single cell from an embryo generated in vitro for genetic testing to diagnose a recurrent, serious, heritable condition and

thereby to avoid the implantation of affected embryos. The technique is used by fertile couples, as an alternative to prenatal diagnosis to avoid the abortion of an affected fetus. However, in the United States, PGD also includes what most Europeans call "preimplantation screening": screening of the embryos of infertile couples for sporadic chromosomal aneuploidies, in order to improve the likelihood of implantation and reduce the risk of miscarriage. In Britain PGD practices are strictly regulated by the Human Fertilisation and Embryology Authority (Braude 2006). The worlds of genetic testing and assisted reproduction have converged with the advent of PGD and debates into different aspects of the technique and its evolving uses will continue.

2.2.2 History of Pre implantation Genetic Diagnosis (PGD)

In 1967, Robert Edwards and David Gardner reported the successful sexing of rabbit blastocysts, setting the first steps towards PGD (Edwards & Gardner 1967a). In many mammalian species, sex chromatin can be identified in a high proportion of interphase nuclei of somatic cells in adult females but not in males. Edward and Gardner suggested that identification of sex chromatin in a high proportion of trophoblast nuclei would allow them to classify these as a female and its relative absence would indicate a male. The sexing of 4-5 day live blastocysts could be useful in excercising some control over the sex ratio of offsprings, as embryos of this age and older can be transferred into receipient females. They also mention that for this vital technique to be of practical use , the embryos must be able to continue normal development and survive to full term after transfer to a recipient female. They had also suggested the possibility of attempts to control sex linked disorders in man using this technique (Edwards & Gardner 1967b).

It was not until the 1980s that human IVF was fully developed, which coincided with the development of the highly sensitive polymerase chain reaction (PCR) method of DNA/gene amplification. Handyside and collaborators' first successful attempts at testing embryos were in October 1989 with the first births in 1990 (Handyside *et al.*.

1992) although the preliminary experiments had been published some years earlier (Coutelle *et al.*. 1989;Handyside *et al.*. 1990a;Holding & Monk 1989). In these first cases of PGD, PCR was used for sex determination for patients carrying X-linked diseases.

PGD has been possible as a result of a number of ground breaking techniques:

i. The ability to fertilise a human egg in vitro and culture the zygote to reach at least day 4 of preimplantation development (Gardner & Lane 1997);
ii. The ability to remove a single blastomere safely from the cleavage stage embryo and also not compromise further development of the embryo (Hardy *et al.*. 1990;Tarin & Handyside 1993);
iii. The ability to amplify minute quantities of DNA using PCR so that genetic analysis can be performed (Sermon *et al.*. 1996;Strom *et al.*. 1994;Tsai 1999).

Since then, its use and popularity has grown steadily. PGD is one of the most rapidly developing techniques in reproductive medicine (Fragouli 2007a).

The following table summarises key developments leading up to IVF and subsequent development:

Table 2.18: Key Development in assissted reproduction

Year	Event	Key Investigators
1880	First attempt at IVF in mammalian eggs	Schenk
1890	First succesful egg recovery from rabbit	Heap
1893	First successful embryo culture	Onanoff
1930	First successful IVF in mammalian eggs resulting in live birth	Pincus
1952	Time of ovulation established in human beings by laparoscopy	Rock
1971	First human blastocyst seen in vitro	Steptoe and Edwards
1972	Mouse embryo successfully cryopreserved and thawed with survival	Wilmut
1978	Birth of Louise Brown, first human born after IVF	Steptoe and Edwards
1983	First pregnancy after replacement of cryopreserved embryo	Trounson
1990	Preimplantation genetic diagnosis first described	Handyside
1992	ICSI developed in human beings	Palermo

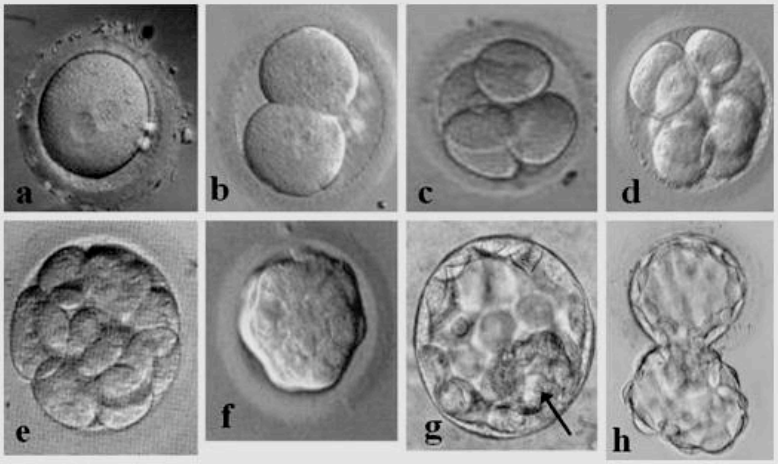
Reproduced with permission from: Sutcliffe AG. IVF children: the first generation—assisted reproduction and child development. London: Parthenon Publishing Group, 2002.

2.2.3 The procedure of preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) is a method by which embryoes formed through IVF can be tested for single gene disorders or chromosome abnormalities prior to embryo transfer. PGD became available only as a consequence of extra corporeal (in vitro) fertilisation. PGD uses standard assisted reproduction techniques such as controlled ovarian stimulation, oocyte retrieval, in vitro fertilisation (IVF) / intracytoplasmic sperm injection (ICSI), and in vitro embryo culture (Ogilvie *et al.* 2005;Pickering *et al.*. 2003). The fertilised egg undergoes reductive cell division (refer to figures 1 & 2) and reaches the 8 celled stage around day 3 post fertilisation. On day 4 morula formation takes place and the embryo reaches the blastocyst stage on day 5.

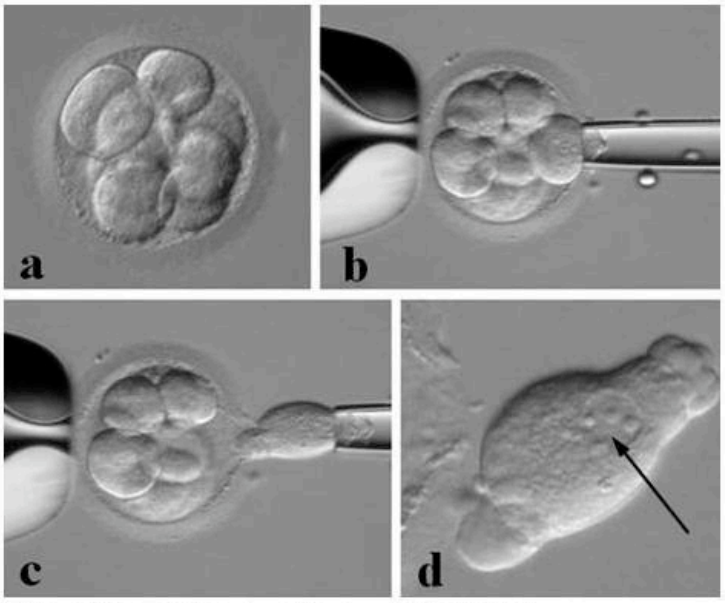
The blastocyst has an inner cell mass which will become the fetus and a clearly differentiated trophectoderm which will form the extraembryonic tissue (Ogilvie *et al.* 2005). PGD uses the biopsy material from either the oocyte or the developing embryo. The biospsied material is tested for the genetic condition in question or screened for chromosomal aberrations and aneuploidy. Only unaffected embryos (no more than two) are transferred to the uterus. The first and second polar bodies may also be biopsied (Rechitsky *et al.*. 1999;Verlinsky *et al.*. 1990;Verlinsky *et al.*. 1998).

Figure 1 Human embryo development from fertilized egg to hatching blastocyst



Ogilvie, C. M. et al. J. Histochem. Cytochem. 2005;53:255-260

Figure 2 Blastomere biopsy of a human cleavage-stage embryo



Ogilvie, C. M. et al. J. Histochem. Cytochem. 2005;53:255-260

This is a labour intensive procedure and can not be used for conditions where the father carries the genetic disorder. However it has the advantage of removing non – functional material and thus is less invasive and damaging than embryo biopsies. The most commonly used technique is to biopsy single blastomeres from embryos on day 3 (De & Van 2001;Veiga *et al.*. 1997). Material for genetic testing may also be taken from the embryo at the blastocyst stage. This yields a larger amount of material for testing than polar body or blastomere biopsy. The disadvantage of this procedure is that the culture conditions may not allow all embryos to progress to blastocyst stage. Also a blastocyst must be transferred to the uterus before hatching on day 6, thus limiting the time available for genetic testing. For genetic testing Polymerase Chain Reaction (PCR), a technique using amplification of specific sequences is used. Alternatively, Fluorescent In Situ Hybridisation (FISH) may be applied to the genetic matrerial (Ogilvie *et al.* 2005). FISH is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescent probes that bind to only those parts of the chromosome which show a high degree of sequence similarity.

2.2.4 Applications of the procedure of Preimplantation genetic diagnosis (PGD)

2.2.4.1 Preimplantation genetic diagnosis (PGD)

PGD was introduced more than 10 years ago to allow couples who had a high risk of having affected children with genetic disease, to avoid the difficult situation of prenatal diagnosis followed by termination of pregnancy (Taylor & Braude 1994). PGD provides information for such couples on which to base their decision - the genetic health of the embryo. It has given these couples the "realistic alternative reproductive option to gamete donation, adoption or continuing to play reproductive roulette" (Braude 2001). PGD was first applied to perform sex selection using PCR for X-linked disease (Handyside *et al.* 1990). It is now offered for a variety of single gene defects (Wells & Sherlock 1998), chromosomal imbalances (Conn *et al.*. 1998;Scriven *et al.*. 2000) and for sex selection for X-linked disease. PGD can be used in any of >1000 genetic tests now available. A number of these indications are

controversial. The following are the general types of PGD testing (Fragouli 2007;Grace *et al.*. 2006;Sermon *et al.*. 2007):

- Autosomal single gene disorders:
    - Autosomal recessive β- thalassemia, cystic fibrosis, spinal muscular atrophy, Tay-Sachs disease, and sickle cell disease.
    - Autosomal dominant Epidermolysis bullosa, Myotonic dystrophy type 1, Huntington's disease, amyloid polyneuropathy, Charcot-Marie-Tooth disease, achondroplasia and Marfan's syndrome.
- Chromosomal rearrangements (inherited chromosomal abnormalities): Robertsonian Translocation (male and female carrier), reciprocal translocation carriers, sex chromosome aneuploidy, pericentric inversion, 21_q 11 deletion
- Specific sex-linked diseases like Duchenne Muscular Dystrophy, Fragile –X syndrome, haemophilia A, Anderson Fabry disease, Incontinentia pigmenti, Alport syndrome, Wiskott- Aldrich syndrome and many more.

2.2.4.2 Preimplantation genetic screening

Preimplantation genetic screening (PGS) also referred to as 'aneuploidy screening', 'low risk PGD' or 'PGD-AS' is carried out for different indications than PGD using different methods. Here instead of single gene probes, FISH is used to look for aneuploidy. PGS is carried out for infertile couples undergoing IVF to prevent the transfer of embryos having obvious chromosomal abnormality/abnormalities, with the hope that this will improve pregnancy outcome. From the report of the 10th Annual Meeting of the International Working Group on Preimplantation Genetics, Italy, June 2000 (Report of 10th Annual meeting, International Working Group of Preimplantation Genetics 2001), one of the major clinical applications of PGD was preselection and transfer of aneuploidy - free oocytes and embryos in cases of assisted reproduction. The Working Group (Report of 10th Annual meeting International Working Group of Preimplantation Genetics 2001) reported more than 1500 clinical cycles with at least 400 clinical pregnancies. Most of these (90%) were

from Chicago, Saint Barnabas and Bologna centres. These three centres have reported the birth of more than 300 healthy babies following the procedure, but there are no details of the assessments of children available from the reports.

To improve the outcome of IVF, aneuploidy testing with PGS is currently being done for an increasing number of indications. They are as following:

- Advanced maternal age
- Poor prognosis women with previous history of chromosomal rearrangement
- Repeated spontaneous abortions
- Multiple IVF failures
- Certain abnormalities of cytoplasmic maturation of oocytes and spermatozoa.

The 10th anniversary report of PGD identified 1500 PGD cycles worldwide for poor prognosis IVF patients, showing that aneuploidy pre selection contribute significantly to improved outcome in pregnancy transfer rate. This was 27% better in the group with advanced maternal age than in younger women. Preliminary follow up study (no details provided) of more than 300 babies born following the procedure confirmed the relative accuracy of preimplantation aneuploidy testing. Several other studies have reported a significant improvement in pregnancy rate. A recent study of women with repeated abortions, by Munne *et al.* reported a pregnancy rate of 50% following PGD compared with a abortion rate of 17% (Munne *et al.*. 2005). Another study by Munne *et al.* showed that PGD significantly reduces pregnancy loss in infertile couples. It also showed that mean pregnancy loss for PGD group was significantly lower compared with the non-PGD group (Munne *et al.*. 2006).

Although there is a steady rise of PGS, the debate about the place and importance of this technique continues (Soini *et al.* 2006). The use of PGS has increased significantly during recent years in IVF cases without any family history of affected offspring (e.g. with no heritable genetic disorder), mainly with the hope of improving the outcome of IVF. This group includes women of advanced maternal

age or cases where embryos have repeatedly failed to implant and women who have had recurrent miscarriages (not related to constitutional chromosomal aberrations) (Soini *et al.* 2006). A recent multi centre, randomised, double blind, controlled trial from Amsterdam reported that PGS did not increase but instead significantly reduced the rate of on going pregnancies and live births after IVF in women with advanced maternal age (Mastenbroek *et al.*. 2007). Shahine and Cedars from San Francisco reported in 2006 that PGD did not increase pregnancy rates in patients at risk for aneuploidy and suggested larger randomised controlled studies before PGD could routinely be recommended as a means of increasing pregnancy rates in patients with advanced maternal age, recurrent pregnancy loss and recurrent IVF failure (Shahine & Cedars 2006).

2.2.4.3. Preimplantation genetic diagnosis-HLA typing

Preimplantation genetic diagnosis (PGD) of single gene disorders, combined with HLA matching, represents an innovation in reproductive medicine. The provision of "saviour siblings" by PGD has been a topic of severe controversy; some criticising this as a step towards "designer babies". PCR protocols are used to provide HLA typing for each embryo. This is done with the intention of matching the embryo to a sick sibling in the family (Verlinsky *et al.*. 2001;Verlinsky *et al.*. 2004b). This embryo is transferred with a hope of establishing pregnancy and thus to provide cord blood stem cells and bone marrow to treat the sick sibling. This approach is particularly valuable for ß-thalassaemia, Fanconi anaemia and other life threatening conditions that require an HLA compatible haematopoietic stemcell donor (Fiorentino *et al.*. 2004). Opinion is divided regarding the appropriateness of this application of PGD. A number of groups have addressed this issue (Fiorentino *et al.* 2004;Rechitsky *et al.*. 2004;Van, V *et al.*. 2004).

2.2.4.4. Preimplantation genetic diagnosis cycles for non-medical (social) sex selection

The report of the ESHRE PGD Consortium data collection VI reported PGD cycles for 'social sexing'. This has given rise to huge controversies (Ray *et al.*. 2002). Sex selection for other than medical reasons has lead to controversy and debate (2003;Baldwin 2005;Dahl 2005;Harris 2005). Attitudes towards pre-conception sex selection for social and family balancing reasons vary amongst cultures. The European convention on Human Rights and Biomedicine, Article 14, explicitly bans sex selection for other than health purposes (1997). Some have raised the question of "reproductive liberty" and question the general ban while they fail to see the moral wrongness of social sexing or family balancing (Dahl 2005). The Human Genetics Commission in the UK has recommended that PGD should not be used for trait or sex selection such that the procedure and its governing bodies could be accused of eugenics.

2.2.5 Results and outcome of Preimplantation Genetic Diagnosis

The reliability of PGD as a diagnostic tool is well established and the error rate in diagnosis though low has been reported (Sermon *et al.*. 2005; Sermon *et al.* 2007). The major drawbacks of PGD are the relatively high cost and that the procedure itself leaves fewer available embryos for transfer (2007). PGD and PGS are still rather rare procedures owing to high technical demands, costs, relative low pregnancy rate and strict licensing in many countries. Very few studies have looked at an integrated analysis of technological, patient related, ethical and economic aspect of the procedures. Results from 10 years data collection from the Netherlands did not report any PGD misdiagnoses, none of the babies had any congenital anomalies and it was suggested that PGD is a reliable and successful method with pregnancy rate similar to IVF and ICSI (de Die-Smulders *et al.*. 2004).

A systematic review from New Zealand reviewing the quantifiable harms and benefits of PGD observed that the incidence and nature of obstetric and neonatal complications after PGD were comparable to those reported after IVF alone. Most

complications were thought related to the risks of multiple gestations (Marjoribanks, Farquhar, & Marshall). The incidence of major birth abnormalities was about 3.8%, similar to that reported after IVF alone. The review concluded that even though PGD appears promising it is important not to overstate its potential.

The 6th report of the ESHRE PGD consortium (Sermon *et al.*, 2007) reported an increasing number of cycles, pregnancies and babies from an increasing number of participating centers. There had been an increase in the number of PGS cycles. At birth PGD babies had characteristics that were similar to those of ICSI babies. No specific complication of pregnancy or malformation was noted in this population. The characteristics of the babies at birth were comparable to and the main complications were related to effects of multiple pregnancies.

2.2.6 Risks of Preimplantation Genetic Diagnosis

PGD uses the usual techniques of ART followed by biopsy of the embryo. As there has been very little follow up of the health and well being of children born after the procedure, there are concerns regarding the safety of the procedure. New ART techniques like the biopsy in PGD and PGS, microinjection in ICSI as well as possible effects from culture media are feared to be detrimental for embryo development and for the health and well being of the children. Many of the existing or possible applications of PGD are at the interface of reproductive medicine and clinical genetics.

A. Risks related to ART

There are ongoing concerns about the possible adverse outcomes in children born after ART. Multiple gestations where both mother and fetus are at risk (Adashi *et al.* 2003), structural anomalies and long term health effects (Bonduelle *et al.* 2005;Hansen *et al.*. 2005;Klemetti *et al.*. 2005) are some of the concerns raised. Several studies on the safety of the techniques have been published, but most have been short term or small. Larger reviews suggested slightly elevated risk of birth defects in children born after ART (Kurinczuk *et al.* 2004).

The concerns about possible adverse effects of ART on children and mothers remain a matter of controversy (Hansen *et al.* 2005). Several studies on adverse effects have been performed, but the methods and patient materials are often not comparable and conclusions have been diverse. The target groups have often been too small to give reliable conclusions (Soini *et al.* 2006) .

Risks to the mother related to multiple gestations included hypertensive disorders, pre-eclampsia, thromboembolism, UTI, anaemia and haemorrhage (placental abruption, placenta previa) and fluid overload in association with parenteral tocolysis (Wennerholm 2003). Risks of still birth and early post natal deaths are increased. Obstetric, neonatal and long term consequences of multiple gestations for the health of ART children are enormous, resulting mostly from premature birth and low birth weight (Wennerholm 2003). Cerebral palsy is one of the most significant neurological impairments associated with multiple births and increases proportionately with the number of fetuses (Wennerholm 2003). This has prompted the reduction of the multiple pregnancy rates to be a high priority for ART programmes. ESHRE has set recommendations from 2000 where this acts as a "criterion of ART excellence" (Hazekamp *et al.*. 2000).

Several studies and reviews have tried to evaluate the risk of adverse effects of ART on the child. The results are partly controversial and also inconclusive (Soini *et al.* 2006). Multiple gestation was clearly a major risk. Many of the other causes of demonstrated adverse effects were unknown, although studies had suggested a link especially with ICSI. Hansen *et al.* concluded that infants conceived following IVF/ICSI are twice as likely to have major birth defects when compared to naturally conceived infants (Hansen *et al.*. 2002). It is not clear why IVF conceived singletons do worse compared to their naturally conceived counterpart in terms of perinatal outcomes (Schieve *et al.* 2002). Data from Danish National registries on obstetric outcomes and neurological sequelae of vanishing twins showed significantly increased risk of preterm birth, low birth weight and cerebral palsy (Pinborg *et al.* 2004). A Finnish study concluded that neonatal outcome after IVF conception is worse compared to general population infants, comparing mothers of similar age,

parity and social class mainly due to the large number of multiple births. The study could not identify the cause of an increased incidence of heart malformations which did not appear to be related to multiple births (Koivurova *et al.*. 2002b).

Review of relevant studies from the US (Hampton 2004) suggested no overall association between ART per se and rates of serious malformations. However the review highlighted that there is:

1) evidence that ART is associated with some adverse neonatal outcomes (low birth weight, perinatal mortality, premature births also in singleton pregnancy);

2) suggestive evidence of an association between ART and congenital imprinting disorders like Angelman syndrome and Beckwith- Wiedemann syndrome.

3) evidence of no association with paediatric cancer or adverse psychological and developmental outcomes.

An international multicentre cohort study was designed to examine both birth defects and mental development in children born after ICSI, IVF and natural conception. The study included 1500 children from five European countries who were followed up to age of 5 years. The study concluded that singleton IVF children were more likely to need health care support when compared to singletons born after natural conception. The overall conclusions were reassuring, apart from the slightly higher rate of congenital abnormalities in ICSI children (Bonduelle *et al.* 2005).

A large Danish study (Lidegaard *et al.* 2005) , reported that childhood cancers, mental diseases, congenital syndromes and developmental disturbances occurred with equal frequencies in a group of 6052 IVF children and 442,349 singleton non IVF children. There was no association between ART and retinoblastoma as had been previously suggested (Moll *et al.*. 2003). However Lidegaard and colleagues reported an 80% increased risk of cerebral palsy amongst IVF children. A Swedish population based study concluded that there was an increased risk for congenital malformations after IVF, regardless of the technique used which is mainly due to parental characteristics (Kallen *et al.* 2005). The safety of ART, which is inherent to PGD, has not been well studied at an epigenetic level. Epigenetic is a phenomenon where modifications of the DNA methylation and/or chromatin structure underlie

changes in gene expression and phenotype characteristics. Disturbances of epigenetic reprogramming may influence gene expression and phenotype characteristics.

There have been concerns about genetic, congenital and developmental abnormalities in children born after transfer of ICSI embryos (Bondulle *et al.* 2004;Braude & Rowell 2003;Golombok 2002).

B. Risks related to embryo biopsy

PGD procedures requiring PCR (defined in section 2.2.3) require ICSI for technical reasons to avoid DNA contamination, whereas in PGD requiring FISH, conventional IVF can be used. Blastomeres at the 4 – 12 cell stage are not identical, but express different regulatory proteins. Certain patterns of fragmentation results in partial or complete loss of regulatory proteins, which in turn affects the developmental potential of the biopsied embryo. Thus, removal of one or two cells from the embryo might theoretically involve a risk of impaired development of the embryo and a potential risk to the offspring. There is very little follow up data from children who were born after PGD/PGS. One study published in 2004 (Verlinsky *et al.*. 2004a), reviewed the 12 year experience of the world's three largest PGD centres and analysed the clinical outcome of PGD in USA and Italy. They reported that more than 6000 clinical cycles had been performed worldwide, resulting in the birth of at least 1000 children. The birth prevalence of congenital malformations in these children was in the range of 5-6% that was not different from population prevalence. There is no data on the outcome of the children born during the 12 years. However a report from Chicago in 2000 (Strom *et al.*. 2000) concluded that data from 109 children born after PGD by polar body removal did not show any detrimental effects of the procedure. Data on perinatal outcome was collected by parental reporting and confirmed by telephone interview and chart review when indicated. If infants were more than 6 months old, a follow up telephone interview was carried out to establish that the babies were appropriately achieving their developmental milestones.

Bonduelle *et al.* have performed many international follow up studies of ICSI (Bonduelle *et al.*. 2003;Bonduelle *et al.*. 2002;Bondulle *et al.* 2004;Sutcliffe *et al.* 2002). The ESHRE PGD Consortium will in future collate data from centres that take care of the complete PGD cycle, from patient referral to transfer and follow up of the babies (Sermon *et al.* 2007). Many professional organisations have stressed the need for systematic long term follow up studies on the children born after ART and PGD. The problem with follow up studies is however that only a minority of parents want to inform their children about the use of ART, which raises issues of informed consent. There needs to be ways to encourage parents to participate in follow up studies relating to health and development of their children (Soini *et al.* 2006).

The development of reproductive sciences and genetics has given a new dimension to ART: as aptly stated in a United Nations Educational, Scientific and Cultural Organisation's (UNESCO) report on PGD and Germ-Line Intervention of 2003, 'IVF aims at having a child, PGD aims at having a healthy child and PGD/human leukocyte antigen (HLA) testing aims at having a healthy and helpful child' (Soini *et al.* 2006). Evidence for the efficacy, reliability and safety of PGS is still limited but growing (Briggs *et al.*. 2000;Sermon *et al.* 2005;Wilton 2002).

2.2.7 Neurodevelopmental and socio-emotional developmental outcome of children born after PGD/PGS

At the beginning of this study there was no follow up data, which had looked into the outcome of children born specifically after PGD/PGS. This was partially because of the fact that PGD still remained a novel technique. Thus there was no report of how PGD/PGS might affect neuro- and socio-emotional development of this group of children.

A recent review by K.J. Middelburg and colleagues (Middelburg *et al.*. 2008) states that the effect of assisted conception on the developing human brain is still not clear, even though the procedures of *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) were introduced more than 25 and 15 years ago, respectively (Palermo *et al.*. 1992;Steptoe & Edwards 1978). Since then many studies have been

conducted on the neurodevelopmental outcome (an umbrella term covering neuromotor, cognitive, speech/ language and behavioural outcome) of children born following ART techniques, but not on newer methods like PGD.

Middelburg and colleagues (Middelburg *et al.*. 2008) reported that in general, the follow-up studies showed no consistent differences in neuromotor, cognitive, language and behavioural development between children born following IVF/ICSI and natural conception. They reported that IVF/ICSI *per se* does not increase the risk for CP, but that an increased risk for CP is related to the association of assisted conception with other risk factors, such as preterm birth. It was also reassuring that no major neurodevelopmental delay or clinically relevant behavioural problems were reported.

Studies looking into the psychological wellbeing of children born following assisted reproductive techniques are many (Colpin *et al.*.1995;Colpin & Soenen 2002;Golombok *et al.*. 2001;Hahn & DiPietro 2001;McMahon *et al.*. 1997;van Balen 1998). Burns (Burns 1990) argued that parents who had difficulty in conceiving might become emotionally over-invested in their long-awaited child, and other authors have suggested that those who become parents after a period of infertility may be overprotective of their children, or may have unrealistic expectations of them, or of themselves as parents (Hahn & DiPietro 2001;McMahon *et al.*. 1997;van Balen 1998). Additionally, it has been predicted that the stress of infertility and its treatment may lead to psychological disorder and marital dysfunction for those who become parents following IVF (McMahon *et al.* 1997).

However a review by Susan Golombok and Fiona MacCallum (2003) (Golombok & MacCallum 2003;McMahon *et al.*. 1997) stated that contrary to the concerns that had been raised regarding the potentially negative consequences of IVF for parenting, studies of these families have generally found IVF parents to be well adjusted and to have good relationships with their children. The review also stated that the children themselves, showed no evidence that they are at risk for cognitive impairment. The social and emotional development of IVF children also appeared to be within the

normal range. In an investigation of the psychological well-being of ICSI children (Place & Englert 2003), the Strengths and Difficulties Questionnaire (Goodman, 1994) was completed by parents and teachers. ICSI children showed no evidence of raised levels of emotional or behavioural problems compared with IVF and naturally conceived children.

Frances Gibson and Catherine McMahon in 2004 (Gibson & McMahon 2004) reported that assisted conception following infertility had little adverse impact on parental wellbeing and parenting skills. The subtle differences identified were not in the realm of clinical problems, but reflected realistic concerns and attitudes in the context of a long and sometimes high-risk path to achieving parenthood. The children appeared to have secure attachment relationships with their parents in childhood and behavioural adjustment was mostly comparable with naturally conceived peers.

A recent report from Belgium by Nekkebroeck and colleagues looked into the mental and developmental outcome of two year old children born after PGD/S (Nekkebroeck *et al.*. 2008). This was very similar to our study but published one month later and thus the second study after ours in the literature. The study concluded that children conceived after PGD/PGS showed similar mental and developmental outcomes at the age of two when compared to children conceived after ICSI or naturally. However this study did not address any child behavioural or parent child/family functioning issues.

This overview sets the scene for the need for the PGD study. The objectives of the study were:

4. To assess a group of PGD/PGS-born children and describe their health in terms of physical/neurodevelopmental and behavioural well being (in relation to a matched control group of children born to parents who did not undergo preimplantation genetic diagnosis).

5. To assess any impact of the difficulties of having a child after PGD/PGS, which can often be stressful for couples, on the psychological health for the parents / or parent-child bonding/ relationships.
6. To consider whether this protocol can then be expanded to a national study of PGD/PGS outcome.

Hypothesis of the study was:

Children born to couples who have had pre-implantation genetic diagnosis are expected to be as healthy when compared to age-matched naturally conceived children. We did not expect to see any of the following effects that will be looked for:

a. Reduced longitudinal growth
b. Greater occurrence of difficult temperament and emotional or behavioural problems
c. Possible effects on neurodevelopment.

Families where children were born post PGD/PGS however may experience the following:

a. Differences in maternal bonding.
b. More stress in parent child relationship.
c. Other difficulties in parenting (related to the pressures of IVF, PGD/PGS and treatment).

Statement of research questions of the study were:

a. What are the physical and neurodevelopmental outcomes for an initial consecutive cohort of these children?
b. Are there difficulties with maternal bonding and parenting skills with these mothers when compared with in relation to matched controls? (Do these mothers experience more difficulties with maternal bonding and parenting skills than their matched controls?

Chapter 3

3. Methods

3.1 Methods of PORD study

3.1.1 Development of the study design and supervision

Study design

This was a population-based (from a hospital database), case-control study carried out in the London. It took approximately one year from the idea for the project to commencement of data collection. In addition to writing a protocol (see Appendix) it was necessary to identify families who were eligible to participate in the study and to obtain ethical approval for the study.

Supervision

It was decided to establish a steering committee for the project, which would consist of my supervisor (AGS), and physicians from the Renal Medicine department at the Royal Free Hampstead NHS Trust (Prof. S. Powis, Clinical Director, Dr. B Thompson, Consultant Physician). The steering committee met three monthly between July 2004 till Jan 2006 to assist and advise on all study matters including recruitment of patients, funding, presentation of findings, technical advice on data collection and ethical matters. Regular monthly meetings were held with my supervisor (AGS) and in addition frequent ad hoc phone calls were necessary with steering committee members on particular issues.

Ethics approval

The Joint UCL/UCLH committees on the Ethics of Human Research (Committee Alpha) granted the study ethics approval. The REC reference number is 05/Q0502/35 dated 9th June 2005.

Power

Power calculation for primary outcome:

Griffith's scales of mental development have a mean score of 100 (and children can be assessed from ages 1 month to 7 years 11 months). We wished to detect a 5 point or more drop in the mean scores with the affected population/group. With a 2 sided test and at a significance level of 0.05

a) An equally sized sample of cases vs. controls requires 99 children per group to achieve 90% confidence.
b) Should there be only 75 recruited per group we would have 80% confidence of a difference.

Power calculation for secondary outcome:

Considering known data on birth weights in children born to mothers with chronic renal disease, we should need to recruit only 10 children per group (case: control) to have 100% confidence of significant difference between the groups on the basis of existing knowledge which suggest a large discrepancy between groups. (C.f. mothers with Chronic Renal Failure: mean birth weight (gms):2239 ± 839 .Mothers on dialysis: mean birth weight (gms) 1543 ± 671. Transplanted mothers: mean birth weight (gms) 2204).

The power of the test, given an effect size corresponding to a 5 point difference between the two groups, with a sample of 24 cases and 37 controls is 0.54. The low power associated with such analysis means that results should be interpreted cautiously.

Statistics

The power analysis for the PORD study indicated that the power to detect any differences or association would be low. The use of individual t-tests and chi-squares was employed as the power of larger multivariate test would be too low. Therefore caution should be exercised in the interpretation of the PORD results as the low power may lead to a Type II error (failing to find a significant effect that exists in the population) and the multiple tests may lead to a Type I error (finding a significant effect due to chance). Any p value of ≤ 0.05 was taken as significant.

3.1.2 Method of Recruitment to the study

The departmental database manager identified from the database of the Renal Department at the Royal Free Hampstead NHS Trust women with renal disease who had reported a pregnancy. The renal service is a combined unit caring for mothers from the Royal Free and Middlesex hospital units. After detailed search using the renal database and hospital PAS system it was first made certain that each women

had a live child as a result of the pregnancy before efforts were made to contact the family. Where there was any doubt about whether the child was alive, the family was not contacted. It was decided to recruit singleton children between their first and eight birthdays. This age range was chosen to allow use of the Griffiths Mental Development Scales for Children aged 0-8 years. It was anticipated that the revised 2-8 year scale of Griffiths Mental Development Scales could be used but due to delay in publication of the scales and validation of data, the study was commenced using the Griffiths 0-2 years (revised 1996) scales and the existing Griffiths 2-8 years scale. After discussion with my supervisor and with his knowledge of the difficulties previously found in recruiting normally conceived control children of higher order births for other studies and because of known confounders which can affect developmental outcome of twins and higher order births it was decided to recruit only singleton children for the PORD study. Qualifying families were contacted by post to obtain written informed consent to participate (or not). Due to Ethics Committee constraints it was not possible to enquire as to why non-participants chose so. Families who did not respond to two written request to participate were considered non-participant. Occasionally families did wish to participate but it was not practicable for them to attend due to work /childcare/related issues. These were not considered further in the study analysis.

3.1.3 Case Definition

a. Children

Children below the ages of 8 years were eligible whose mothers were known to have chronic kidney disease or who had a renal transplant at the point of positive pregnancy test.

b. Renal disease in Mothers

Chronic kidney disease in mothers was be categorised as Stages 1 - 5 depending upon their glomerular filtration rate as per the US National Kidney Foundation Kidney Disease Quality Outcomes Initiative classification of stages of chronic kidney disease (Nation Kidney Foundation , clinical practice guidelines 2002b)

Table 3.1 Stages of Chronic renal failure

Stage	Description	Glomerular filtration rate (GFR) in ml/min/1.73m^2
1	Kidney damage with normal or high GFR	≥ 90
2	Kidney damage with slightly low GFR	60 - 89
3	Moderately low GFR	30 – 59
4	Severe low GFR	15 – 29
5	Kidney failure	< 15 or dialysis

Chronic kidney disease is defined as either kidney damage or glomerular filtration rate <60 ml/min/1.73m^2 for ≥3 months. Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies.

From our database review we had estimated that maternal cases were split across stages 1-5 approximately equally. However, during the study it was evident that it would be difficult to classify study cases as above mainly due to lack of information from case notes and also due to the fact that all mothers were not screened or followed up in the same manner. Thus we divided them into categories of renal clinic attended i.e. general nephrology, renal transplant and chronic renal failure.

c. Study families

All eligible families were contacted using a standard letter approved by the local ethics committee and derived from the study protocol (appendix).

From our combined database an estimate was made of at least 150 families with children less than eight years who could be potentially recruited for the study.

Out of an original cohort of 150 qualifying children, only 80 living within the London region were eligible for the study. The other 70 families were not eligible as the child/children were over 8years or the family had moved and was no longer in London and logistically it was difficult to organise participation. Of the 80, a total of 35 (44%) responded to the invitations and finally 24 (30% of eligible children) born between August 1997 and October 2005 were subsequently seen for assessment.

d. Controls

In order to attain a one to one ratio of study to control children, the local child health database was used to recruit control children. Children were identified from the Regional Interactive Child Health System (RICHS) which was a community database system of some 500,000 children then used by 9 North – East London Primary Care Trusts (PCTs). The details kept included: demographic; birth; immunisations; examinations; medical details e.g. ICD 10 codes and health professional involvement. Control families were contacted using a standard letter approved by the local ethics committee and derived from the study protocol (appendix). Two control children were approached for each index case, as it is anticipated there would only be 50% participation rate amongst approached controls. Written consent was obtained from all participating control families and they were assessed in an identical manner to study children.

3.1.4 Exclusion and inclusion criteria for children in the existing database

a. Exclusion criteria

Children aged over 8 years, children whose mother tongue was not English and families not living in mainland UK were not recruited; as they were not eligible for Griffiths Mental Development Testing (this has an upper age limit of 7 yrs 11 months).

b. Inclusion criteria:

Children recruited were singleton and born to mothers with chronic renal disease. (See above)

3.1.5 Matching

Control children were matched with cases for age, sex, social class (according to Office of National Statistics classification), gestational maturity and race. All children were singleton.

3.1.6 Benefits of assessment

Families who agreed to take part in the study were given the opportunity to discuss medical and behavioural concerns with the paediatrician at the time of assessment.

The families were also advised that they could contact the investigators at any time in the future if there were any further concerns of a paediatric nature, regardless of relevance to the study *per se.*

During the course of the assessment, no previously unrecognised medical problems were discovered. However, many parents discussed concerns sometimes behavioural, which were addressed to the best ability of the paediatrician and if necessary appropriate referrals were initiated. This was perceived as very helpful to the parents who often were in doubt about the nature of the problem and also the appropriateness of referral to other services.

3.1.7 Data collection and recording

A data collection form was designed. In order to make data entry systematic and more concise a series of fixed numeric lists were designed enabling most collected information to be entered in numeric form (see appendix). Arrangements were made to enter raw data into a computer database for subsequent analysis. An appropriate data input programme using File Maker Pro version 5 was designed by Hui Jayne Lim (Research assistant) after piloting the format on 6 cases. It was necessary to develop standardised criteria for this data input (see Appendix). This method of standardised data collection allowed later statistical analyses more readily to be performed. Data was transferred and repeatedly backed up in the Medical School's computer Centre. Data were initially collected with individual identifiers but stored for analysis in an anonymised format.

The following data were collected:

a. Socio-demographic details

For each participating biological parent the following details were obtained:

- Age
- Marital status was recorded according to category: married, single, divorced, separated or cohabiting.

- Parent's educational level categorised into no formal qualifications; GCSE or equivalent; A-level or equivalent; University entry or equivalent; Degree and Higher degree.
- Occupations
- Social class was then ascertained according to the Office of National Statistics classification. This is a scale for classifying people into five groups (represented by roman numerals), one subdivided, based on occupation. (Formerly Registrar General's Social Class).
- Smoking categorised as non-smoker, 1-5 cigarettes per day, 6-10 cigarettes per day, 11-20 cigarettes per day, 20+ cigarettes per day, or pipe/cigar smoker.
- Drinking categorised as units per week.

In all the above an unknown category was allowable.

b. Perinatal details

Details of maternal illnesses, obstetric complications and medications taken during pregnancy were also recorded.

Mothers were asked if they smoked or drank alcohol during the pregnancy; if so, the amount was quantified. Alcohol consumption was recorded as the number of units consumed per week.

Other data obtained included mother's intention to breast feed and the number of persons providing adult support for mother during the pregnancy.

Details of delivery included the date of delivery, gestation at delivery in weeks and days; whether the labour commenced spontaneously or required induction or, as in some cases, there was no labour (elective caesarean section); and the type of delivery-classified as normal vaginal, forceps/ventouse, planned caesarean or emergency caesarean section and unknown

c. Neonatal details

The following were recorded: sex, birth weight in grams, birth length in cms, birth head circumference in cms and Apgar scores obtained from the parent held Personal

child health record. If details were missing, and where possible information was obtained from other parent-provided sources.

Records were made of the need for resuscitation at birth and for admission to the neonatal unit. If admission to a neonatal unit was required, then the length of stay and the reason(s) for admission were recorded. The need for and length of ventilation if any was recorded. Neonatal illnesses were recorded using a defined list and congenital anomalies were classified as major or minor using ICD 10 (International Classification of Diseases 10, World Health Organisation, Geneva 1992). Minor defects were considered to be those that posed no significant health or social burdens and major defects were those that could adversely affect the child's health or development.

The type of feeding was recorded as exclusive breast feeding, formula or mixed. The length of exclusive breast feeding was noted. The number of adults providing support for the baby and family was recorded including parents.

3.1.8 The assessment

A single paediatrician (IB) assessed all the children using a standardised protocol. The children were assessed in a child friendly environment. A quiet room (within the Royal Free and University College Medical School) was used to assess the child and interview the parent(s). The child's height, weight, occipito frontal head circumference (for children up to 5 years) and blood pressure were measured in the out patient clinic of the Department of Paediatrics and Child Health, Royal Free Hampstead NHS trust. The physical assessment was also carried out in the same place in a dedicated clinic room. This was followed by the developmental assessment, which involved the administration of the Griffiths Mental Development Scales.

After the child's assessment the paediatrician completed a general medical questionnaire during interview with the parents. This included socio-demographic details and past medical history including perinatal, neonatal and childhood data. The paediatrician also recorded all physical findings on this form.

3.1.8.1 Physical assessment

a. General physical examination

A general medical examination was performed on all the children. This included cardiovascular, respiratory, ear nose and throat, abdominal and skin assessment.

Neurological examination including gross motor skills was also performed. Attention was also paid to the dentition, hair and skeleton. The genitalia were examined. Testicular descent was checked for in male children.

b. Congenital anomalies

The children were examined carefully for evidence of congenital abnormalities and if found these were recorded according to the International Classification of Diseases (ICD-10)

c. Growth

All children were measured for height, weight and occipito-frontal head circumference. Height was measured using a portable stadiometer recommended by the Child Growth Foundation. Weight was measured using electronic scales. Centiles for height and weight were then calculated using a computer programme (L-grow®), standardised for a UK cohort of children (LGROW 1998)

If the child co-operated the blood pressure was measured using Dinamap™ Compact T model of automated BP measuring instruments (standardised for use in children of all weights/ages). However, it was soon evident that quite a few younger children did not want this done.

d. Audiometry and vision

It was planned to test hearing of each child using a portable pure tone audiometer. Results were recorded as normal, abnormal, no cooperation, difficult to assess, not done. Distance vision was examined in children over 5 years using Snellens charts. Squints were examined by using the cover test.

However it was soon evident that most children didn't, co-cooperate with the hearing and vision tests. There were also difficulties with the noise levels in the rooms used for assessments. Thus parents were asked about any concerns relating to hearing and vision.

3.1.8.2 Psychological assessments

The age appropriate Griffiths Mental Development Scales (Griffiths 1996) was administered to all children in the study.

3.1.8.3 Parental questionnaires to assess family functioning

The mothers of all participating children were asked to complete four questionnaires relating to family functioning. The questionnaires were posted to the family along with the appointment letter for the assessment. The mothers were requested to complete the questionnaires at home. The parents were provided with pre paid reply envelope to return the questionnaire or they brought them along on the day of the

assessment. The psychologist scored the returned questionnaires and the numerical scores were added to the computer database. If the questionnaires were not returned, a reminder was sent to the parents with a second copy of the questionnaires. Copies of the questionnaires are provided in the appendix

The following questionnaires were completed by the mothers:

1. Child Behaviour Checklist for ages 1½-5 and ages 6-18 (Achenbach & Rescorla 2001)
2. The Carey Temperament Scales (Second Edition)(Carey 2005)
3. The parenting Stress Index /Short form (Abidin 1990)(Abidin 1990)
4. The Parental Acceptance and Rejection Questionnaire (Rohner and Khaleque 2005)(2005b)
5. General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)(Goldberg & Hillier 1979b)

The above tools were used after discussion with Prof Barnes: Psychological supervisor to the project. These tools have been used in a very similar study conducted in the department previously (Peters 2005) and also in European multicentre studies (Barnes *et al.* 2004). Even though the scales were American they have been used in several Europan studies with ease and consistent results. However, there does remain the concern of using the tools in a UK based population study. Some of the questionnaires were lengthy with the print often unfriendly; however none of them took more that 15-20 minutes tocomplete.

3.1.9 Description of measures used

a. The Griffiths Mental Development Scales

Each child was tested using the revised Griffiths Mental Development Scales from birth to 2 years (1996 Revision) and the Griffiths Mental Development Scales from birth to eight years (1984). The Griffiths Mental Development Scales from birth to 2 years re-standardised in the United Kingdom in 1996 to reflect the child rearing practices, social habits, racial groupings and other socio-demographic factors pertinent to modern day Britain. The extended 2-8 years Griffiths scales had been undergoing an update with re-standardising and validation. This process was taking much longer than expected, so these scales could not be used in this study. The Griffiths Mental Development Scales (GMDS 1954, 1979, 1984) were developed by Dr. Ruth Griffiths to assess the developmental level of children from birth to two years of age on five subscales, namely:

- Subscale A: Locomotor
- Subscale B: Personal-social
- Subscale C: Hearing and Language
- Subscale D: Eye and Hand Co-ordination
- Subscale E: Performance

During the 1960s the scales were expanded to cover from birth to eight years (Griffiths 1970) and a sixth subscale was added

1. Subscale F: Practical Reasoning

The scales have full stringent statistical requirements of reliability and validity. In the GMDS for 0-2 years each subscale is based on a mean of 100 and S.D. of 16 and the total scale is based on a mean of 100 and S.D. of 12. The GMDS for birth to eight year the total of scales have a General Quotient of 100.18 and S.D. of 12.76.

b. Child Behaviour Checklist for ages 1½-5 and ages 6-18

The CBCL/1½ - 5 a revision of the CBCL/2-3 (Achenbach, 1992) – can be completed by parents, parent surrogates or others who see a child in a family setting. The check -list requests demographic information about the child and asks

respondents to indicate their name and their relationship to the child, such as mother, father, foster parent or other relationship. Parent's occupation is noted. The respondent is then asked to rate 99 problem items as *0* for *not true* of the child, *1* for *somewhat or sometimes true*, and *2* for *very true or often true,* based on the preceding two months. For several items, respondents are asked to provide descriptions of the problems. In addition item 100 requests respondents to write any additional problems that were not previously listed.

Following the items to be rated, the CBCL/1½- 5 provides open-ended items that ask the respondent to describe any illnesses or disabilities that the child has, what concerns the respondent most about the child and the best things about the child. The responders thus provide not only ratings that are scored on the scales to be described later but also descriptive information specific to the child who is being assessed. The scales are designated as Syndrome Scales of Emotionally Reactive, Anxious/Depressed, Somatic Complaints, Withdrawn, Sleep Problems, Attention Problems and Aggressive Behaviour. In addition to the syndrome scale, the CBCL 1½- 5 can be scored in terms of two broad groupings of syndromes designated as *Internalising (four syndromes on the left side of the profile)* because it comprises problems that are mainly within the self and *Externalising (two syndrome on the right hand side of the profile)* because it comprises problems that mainly involve conflicts with other people and their expectations of the child.

The CBCL/6- 18 is a revision of the CBCL/4-18 (Achenbach 1991). It is completed by parents, parent surrogates or others who see a child in a family setting. The list requests demographic information about the child and asks respondents to indicate their name and their relationship to the child, such as mother, father, foster parent or other relationship. Parent's occupation is noted. The respondent rates each problem item as *0 = not true, 1= somewhat or sometimes true* and *2= very true or often true* based oh the preceding 6 months. The respondent then completes the competence items on pages 1 and 2, followed on page 2 by open ended items for describing the child's illnesses and disabilities, what concerns the respondent most about the child and the best things about the child. The responders thus provide not only ratings that are scored on the scales to be described later but also descriptive information specific

to the child who is being assessed. The CBCL/6-18 identifies the competencies and problems reported for the child. The competence scales are designated as activities, social and school scales which add up to CBCL total competence score.

The CBCL /6-18 also have syndrome scales comprising of the problem items. The scales are designated as Anxious/Depressed, Withdrawn/Depressed, Somatic Complaints, Social Problems, Thought Problems, Attention Problems, Rule Breaking Behaviour and Aggressive Behaviour.

In addition to the syndrome scale, the CBCL/6-18can be scored in terms of two broad groupings of syndromes designated as *Internalising (three syndromes on the left side of the profile)* because it comprises problems that are mainly within the self and *Externalising (two syndrome on the right hand side of the profile)* because it comprises problems that mainly involve conflicts with other people and their expectations for the child.

c. The Carey Temperament Scales (Second Edition)

The Carey Temperament scales comprise a series of behavioural rating instruments developed by William B Carey and associates for assessing temperamental characteristics in infants and children up to the age of 12 years. The questionnaire is a measure of nine NYLS (New York Longitudinal Study) temperament characteristics: activity level, rhythmicity, approach-withdrawal, adaptability, intensity, mood, attention span and persistence, distractibility and sensory threshold.

The questionnaire contains up to 100 items, each rated on a 6 point scale of frequency ranging from almost never to almost always. When tabulated the items scores yield a category score for each of the nine areas which can be compared to the norms for the category. In addition general perceptions for each category are included and compared to the ratings as well as an overall impression of difficulty. Finally, diagnostic clusters of easy, intermediate low, slow to warm up, intermediate high and difficult are made based on the overall profile seen in the questionnaire.

The following questionnaires were used

1. Early Infancy Temperament Questionnaire for 1 to 4 month old infants.

2. Revised Infant Temperament Questionnaire for 4 to 11 month old infants.
3. Toddler Temperament Scale for 1-2 year old children.

Although the questionnaires assess the same temperament characteristics at each level, the situations (context) in which the items are rated are changed to be appropriate for the child's developmental level. Because of the psychometric criteria for inclusion, items were rejected if they did not correlate with their assigned scales for any given age level.

d. The parenting Stress Index /Short form (Abidin 1990)

This questionnaire is based on the theory that the three measured subscales influence parenting behaviour.

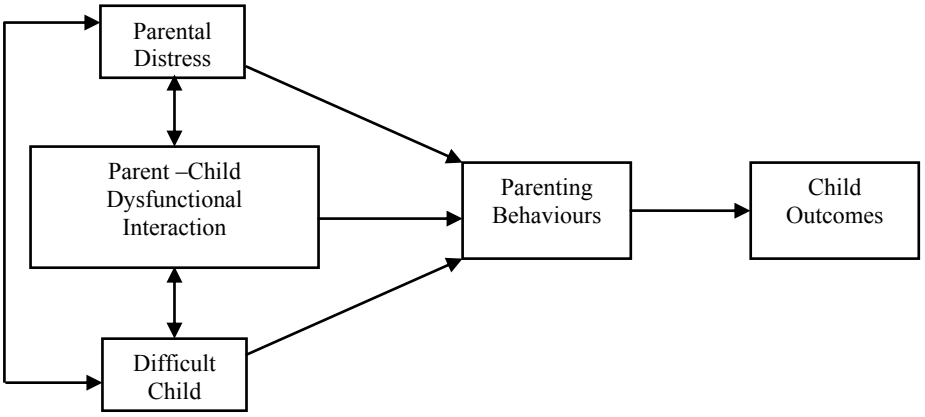


Figure 3.1: Theoretical Model for the Parenting Stress Index (Abidin 1990)

The questionnaire included 36 items with a 5-point agree/disagree response scale. The responses measure the three subscales of the Parental Distress, Parent-Child Dysfunctional Interaction and Difficult Child Behaviour. The sum of these three scores represents the total parenting stress score. Parents with a total score above 90th percentile have clinically significant levels of stress.

This questionnaire also includes a defensive reporting scale. This is to assess possible parental bias in their responses. A parent with an extremely low score is either trying to portray a very competent individual or is detached in their role as parent or is an extremely competent parent.

e. The Parental Acceptance and Rejection Questionnaire (Rohner and Khaleque 2005)

Parental acceptance-rejection theory (PARTheory) is an evidence based theory of socialisation and lifespan development that attempts to predict and explain major causes, consequences and other correlates of parental acceptance and rejection within the United States and worldwide. It attempts to answer 5 classes of questions divided into 3 sub theories: personality, coping and socio-cultural sub theory.

The Parental Acceptance-Rejection Questionnaire (PARQ) is a self-report instrument designed to measure individuals' perceptions of parental acceptance (i.e. the warmth dimension of parenting). It is a bipolar dimension, with acceptance defining one end of the continuum and parental rejection defining the other.

All versions of PARQ consist of four scales:

1. Warmth and affection
2. Hostility/aggression
3. Indifference /neglect
4. Undifferentiated neglect

All versions and forms of PARQ are nearly identical except for verb tense (present or past tense) and referent (father or mother version of the PARQ). We used the parent PARQ and the adults were told to reflect on the way they now treat their children. Respondents were told not to dwell for any length of time on any given item. The object of this test is to get respondents' first overall reaction. The respondents were reminded that there is no right or wrong answer to any item. The respondent should ideally complete it in one sitting without distraction. It takes between 10-15 minutes to complete the questionnaire.

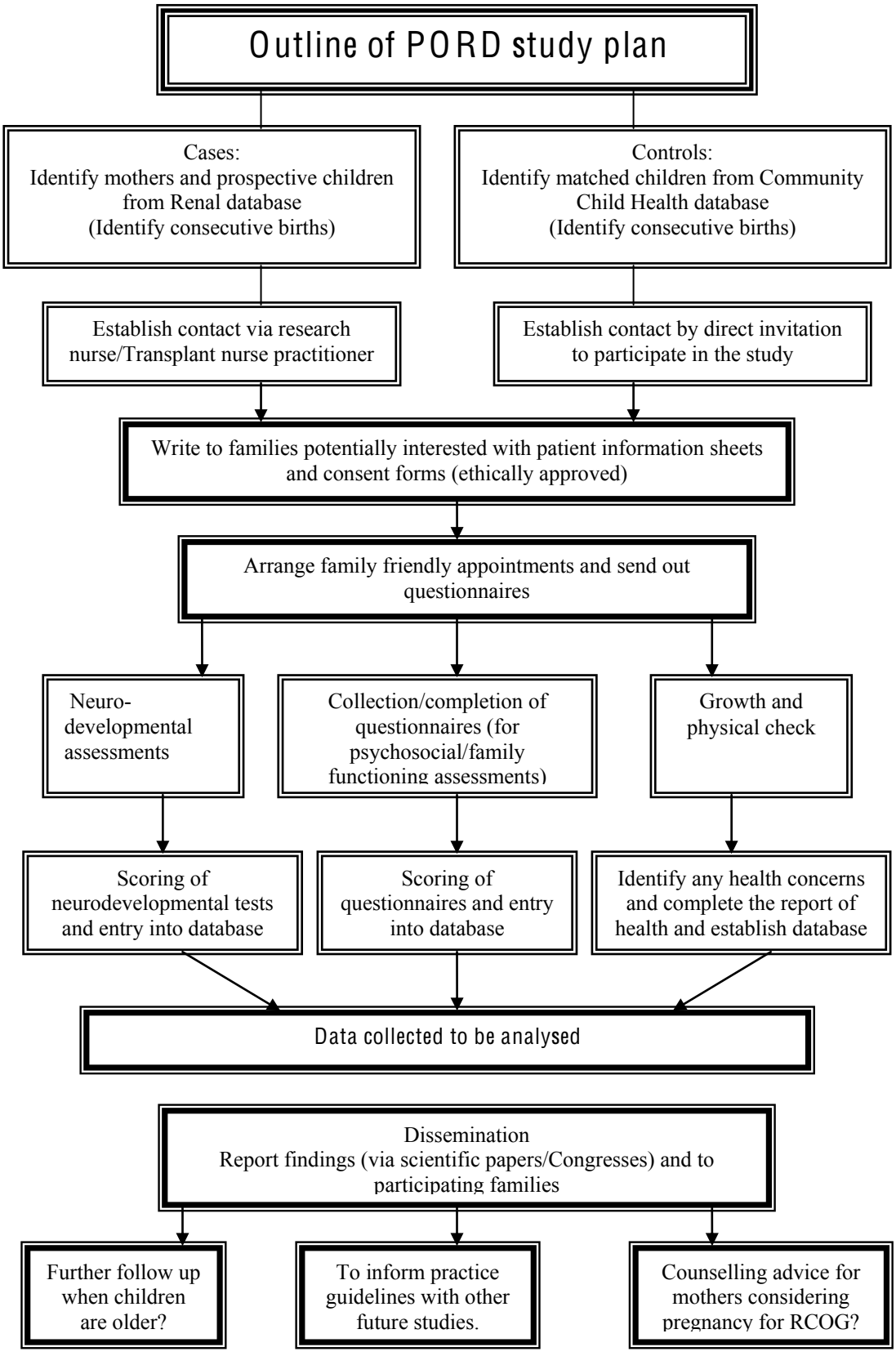
The questionnaire uses the following response format: *Almost always true, Sometimes true, Rarely true, Almost never true.* In order to minimize yea-saying/nay-saying and other forms of response set bias, nearly half the items on the standard PARQ must be reverse scored to create a total acceptance rejection score.

f. General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)

This is a self administered 28 item screening instrument designed to detect current mental health problems in the general population. The GHQ-28 provides four dimensions, measuring somatic symptoms, anxiety and insomnia, social dysfunction and severe depression.

Summary

The assessment protocol is summarised in the following flow chart:


Outline of PORD study plan
Cases:
Identify mothers and prospective children from Renal database
(Identify consecutive births)
Controls:
Identify matched children from Community Child Health database
(Identify consecutive births)
Establish contact via research nurse/Transplant nurse practitioner
Establish contact by direct invitation to participate in the study
Write to families potentially interested with patient information sheets and consent forms (ethically approved)
Arrange family friendly appointments and send out questionnaires
Neuro-developmental assessments
Collection/completion of questionnaires (for psychosocial/family functioning assessments)
Growth and physical check
Scoring of neurodevelopmental tests and entry into database
Scoring of questionnaires and entry into database
Identify any health concerns and complete the report of health and establish database
Data collected to be analysed
Dissemination
Report findings (via scientific papers/Congresses) and to participating families
Further follow up when children are older?
To inform practice guidelines with other future studies.
Counselling advice for mothers considering pregnancy for RCOG?

3.2 Methods of PGD study

3.2.1 Development of the study design and supervision

Study design

This was a population-based (from fertility clinics), case-control study carried out in the London area. It took approximately six months from the idea for the project to commencement of data collection. In addition to writing a protocol (see Appendix) it was necessary to identify families who were eligible to participate in the study and to obtain ethical approval for the study.

Five centres with established PGD programmes in London were contacted for agreement to participate. Of these five centres, four agreed to help recruit patients.

Supervision

It was decided to establish a steering committee for the project, which would consist of my supervisor (AGS), and obstetricians from the four participating PGD centres which are the following:

1. Mr. M Taranissi: Consultant, Assisted Reproductive and Gynaecology Centre
2. Prof. G. Grudzinskas : The London Bridge Fertility, Gynaecology and Genetics Centre
3. Mr. H Abdalla: Consultant, Lister Fertility Clinic, Lister Hospital
4. Mr. O Ozturk: Consultant, The Assisted Conception Unit, University College Hospital,

The steering committee has met three monthly between July 2006 till June 2007 to assist and advise with all study matters including recruitment of patients, funding, presentation of findings, technical advice on data collection and ethical matters. Regular monthly meetings were held with my supervisor (AGS) and in addition frequent ad hoc phone calls were necessary with steering committee members on particular issues.

Ethics approval

The Joint UCL/UCLH committees on the Ethics of Human Research (Committee A) granted the study ethics approval. The REC reference number is 06/Q0505/27 dated 23rd August 2005.

Power

Power calculation for primary outcome:

Griffith's scales of mental development have a mean score of 100 and each of the five subscales have a mean value of 100 and a SD of 10.8 (and children can be assessed from ages 1 month to 24 months). The power calculation was done at the conception of the study as follows:

We wished to detect a 5 point or more drop in the mean scores with the affected population/group. With a 2 sided test and at a significance level of 0.05

a. An equally sized sample of cases vs. controls required 99 children per group to achieve 90% power to detect a difference.
b. Should there be only 75 recruited per group we would have 80% power to detect a difference.

The power of the test, given an effect size corresponding to a 5 point difference between the two groups, with a sample of 49 cases and 66 controls is 0.78.

Statistics

Statistical analyses were conducted using SPSS for Windows 15.0. Between group differences on continuous variables were analysed using t-tests and means and standard deviations are reported. Chi-square tests were used for categorical variables and frequencies and percentages are reported. Where continuous variables were expected to be highly correlated, such as separate subscales, multivariate analysis of variance (MANOVA) was used and the between–groups effects were reported. MANOVA was used to for the analysis of the subscales of the Griffiths Scale, the Parenting Stress Index, the Parental Acceptance-Rejection Questionnaire, and the Toddler Temperament Questionnaire. The omnibus test was used to determine if there were overall effects, and between-subjects tests were used to identify those variables where differences were.

3.2.2 Method of Recruitment to the study

The respective centres identified families who would be eligible to participate in the study. To preserve confidentiality the parents were initially contacted by their PGD

clinic and were requested to write to the researchers indicating willingness to participate. Once the families had responded indicating their willingness to participate, they were contacted by a research assistant in the Department of Child Health offering appointments suitable for the family. It was decided to recruit children between their first and fifth birthdays. This age range was chosen to allow use of the Griffiths Mental Development Scales for Children aged 0-8 years and it was also evident from the responses received that most children born after PGD were within this age range (ages of 0-5 years). It was anticipated that the revised 0-8 year scale of Griffiths Mental Development Scales could be used but due to delay in publications of the scales and validation of data, the study was commenced using the Griffiths 0-2 years (revised 1996) scales and Griffiths 2-8 years scale. This was done to avoid delay in assessments of children.

There were some families who responded indicating their unwillingness to participate. Due to Ethics Committee constraints it was not possible to inquire as to why non-participants chose so. Families who did not respond to two written request to participate were considered non-participant. Occasionally families did wish to participate but it was not practicable for them to attend due to work /childcare related issues. These were not considered further in the data analysis.

3.2.3 Case Definition

a. Children

Children below the ages of 8 years who were born following preimplantation genetic screening or diagnosis were eligible to participate.

b. Study families

All eligible families were contacted using a standard letter approved by the local ethics committee and derived from the study protocol (appendix).

A combined estimate from the four PGD centres suggested there were at least 100 children less than eight years who could potentially be recruited for the study. Of an original cohort of 100 qualifying children, only 70 living within the London Region or just outside were eligible for the study. Of these 70 a total of 49 (70%) born between October 2002 and January 2007 were subsequently seen for assessment.

c. Controls

Naturally conceived children were recruited from local nurseries to provide a control group. The London based Multiple Birth Foundation was approached for recruitment of twin controls. Children who had emigrated, who were born less than 32 weeks gestation or whose mother tongue was not English were not approached. Control families were contacted using a standard letter approved by the local ethics committee and derived from the study protocol (appendix). Two control children were approached for each index case, as it was anticipated there would be only 50% participation rate amongst potential controls. Written consent was obtained from all participating control families and they were assessed in an identical manner to study children.

3.2.4 Exclusion and inclusion criteria for children in the existing database

a. Exclusion criteria

Children aged over 8 years were not recruited; as they were not eligible for Griffiths Mental Development Testing (this has an upper age limit of 7 yrs 11 months). They were also excluded if their mother tongue was not English or they were no longer living in mainland United Kingdom.

b. Inclusion criteria

Children who were born following Pre implantation genetic diagnosis or screening were included.

3.2.5 Matching

Control children were matched with cases for age, sex, social class (according to Office of National Statistics classification), gestational maturity and race.

3.2.6 Benefits of assessment

Families who agreed to take part in the study were given the opportunity to discuss medical and behavioural concerns with the paediatrician at the time of assessment. The families were also advised that they could contact the investigators at any time in

the future if there were any further concerns of a paediatric nature, regardless of relevance to the study *per se*.

During the course of the assessment, no previously unrecognised medical problems were discovered. However, many parents discussed concerns sometimes behavioural, which were addressed to the best ability of the paediatrician and if necessary appropriate referrals were initiated. This was perceived as very helpful to the parents who often were in doubt about the nature of the problem and also the appropriateness of referral to other services.

3.2.7 Data collection and recording

A data collection form was designed. In order to make data entry systematic and more concise a series of fixed numeric lists were developed enabling most collected information to be entered in numeric form (see appendix). Arrangements were made to enter raw data into a computer database for subsequent analysis. An appropriate data input programme using File Maker Pro version 5 was designed by Hui Jayne Lim (Research assistant) after piloting the format on 6 cases. It was necessary to develop standardised criteria for this data input. This method of standardised data collection allowed later statistical analyses more readily to be performed. Data were transferred and repeatedly backed up in the Medical School's computer centre. Data was initially collected with individual identifiers but stored for analysis in an anonymised format.

The following data were collected:

1. *Socio-demographic details*

For each participating biological parent the following details were obtained:

- Age
- Marital status was recorded according to category: married, single, divorced, separated or cohabiting.
- Parent's educational level categorised into no formal qualifications; GCSE or equivalent; A-level or equivalent; University entry or equivalent; Degree and Higher degree.
- Occupations
- Social class was then ascertained according to the Office of National Statistics classification. This is a scale for classifying people into five groups

(represented by roman numerals), one subdivided, based on occupation. (Formerly Registrar General's Social Class).

- Smoking categorised as non-smoker, 1-5 cigarettes per day, 6-10 cigarettes per day, 11-20 cigarettes per day, 20+ cigarettes per day, or pipe/cigar smoker.
- Drinking categorised as units per week.

In all the above an unknown category was allowable.

2. Perinatal details

Details of maternal illnesses, obstetric complications and medications taken during pregnancy were recorded.

Mothers were asked if they smoked or drank alcohol during the pregnancy and if so, the amount was quantified. Alcohol consumption was recorded as the number of units consumed per week.

Other data obtained included mother's intention to breast feed and the number of persons providing adult support for mother during the pregnancy.

Details of delivery included the date of delivery, gestation at delivery in weeks and days; whether the labour commenced spontaneously or required induction or, as in some cases, there was no labour (elective caesarean section); and the type of delivery-classified as normal vaginal, forceps/ventouse, planned caesarean or emergency caesarean section and unknown.

3. Neonatal details

Records of the child's sex, birth weight in grams, birth length in cms, birth head circumference in cms and Apgar scores were obtained from the parent held child record. If the details were missing, the parents were asked if they had other records from which this information could be obtained.

Records were made of the need for resuscitation at birth and for admission to a neonatal unit. If admission to a neonatal unit was required, then the length of stay and the reason for admission were recorded. The need for and length of ventilation was recorded. Neonatal illnesses were recorded using a defined list and congenital anomalies were classified as major or minor using ICD 10 (International

Classification of Diseases 10, World Health Organisation, Geneva 1992). Minor defects were considered to be those that posed no significant health or social burdens and major defects were those that could adversely affect the child's health or development.

The type of feeding was recorded as exclusive breast feeding, formula or mixed. The duration of exclusive breast feeding was noted. The number of adults providing support for the baby and family was recorded including parents.

3.2.8 The assessment

Children who were aged up to 5 years and their parents were assessed by a single observer (IB) blinded to the child's conception status in a child friendly outpatient setting, using a standardised protocol. Blinding was achieved by a separate researcher approaching families for recruitment. The informed consent process also involved forewarning families not to reveal the child's conception status to the paediatrician. The child's height, weight and occipito frontal head circumference (for children up to 5 years) were measured in the out patient clinic of the Department of Paediatrics and Child Health, University College Hospitals NHS Trust. The physical assessment was also carried out in an adjacent dedicated clinic room. This was followed by the developmental assessment, which involved the administration of the Griffiths Mental Development Scales.

After the child's assessment the paediatrician completed a general medical questionnaire during interview with the parents. This included socio-demographic details and past medical history including perinatal, neonatal and childhood data. The paediatrician also recorded physical findings on this form.

3.2.8.1 Physical assessment

a. General physical examination

A general medical examination was performed on all the children. This included cardiovascular, respiratory, ear nose and throat, abdominal and skin assessment.

Neurological examination including gross motor skills was also performed. Attention was also paid to the dentition, hair and skeleton. The genitalia were examined in the children. Testicular descent was checked for in male children.

b. Congenital anomalies

The children were examined carefully for evidence of congenital abnormalities and if found these were recorded according to the International Classification of Diseases (ICD-10)

c. Growth

All children were measured for height, weight, occipito-frontal circumference. Height was measured using a portable stadiometer recommended by the Child Growth Foundation. Weight was measured using electronic scales. Centiles for height and weight were then calculated using a computer programme (L-grow®), standardised for a UK cohort of children (LGROW 1998)

3.2.8.2 Psychological assessments

Each child was tested using the Griffiths Mental Development scales.

3.2.8.3 Parental questionnaires to assess family functioning

The mothers of all participating children were asked to complete four questionnaires relating to family functioning. The questionnaires were posted to the family along with the appointment letter for the assessment. The mothers were requested to complete the questionnaires at home. The parents were provided with a pre paid reply envelope to return the questionnaires or they brought them along on the day of the assessment. The psychologist scored the returned questionnaires and the numerical scores were added to the computer database. If the questionnaires were not returned, a reminder was sent to the parents with a second copy of the questionnaires. Copies of the questionnaires are provided in the appendix

The following questionnaires were completed by the parents:

1. Child Behaviour Checklist for ages 1½-5(Achenbach & Rescorla 2000)
2. The Carey Temperament Scales (Second Edition)(Carey 2005)

3. The parenting Stress Index /Short form (Abidin 1990)(Abidin 1990)
4. The Parental Acceptance and Rejection Questionnaire (Rohner and Khaleque 2005)(2005a)
5. General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)(Goldberg & Hillier 1979a)

3.2.9 Description of measures used

The Griffiths Mental Development Scales

Child Behaviour Checklist for ages 1½-5

The Carey Temperament Scales (Second Edition)

The parenting Stress Index /Short form (Abidin 1990)

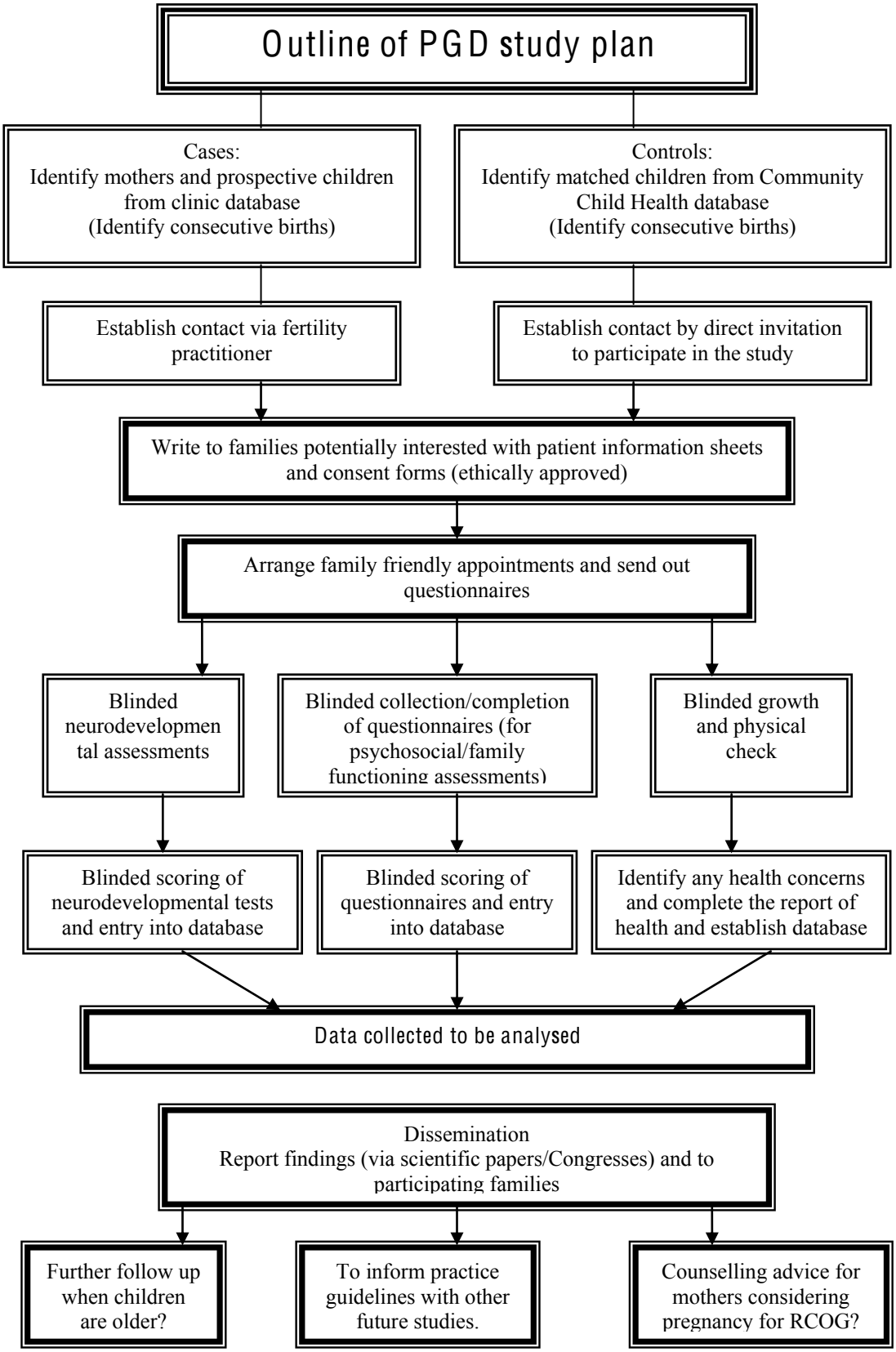
The Parental Acceptance and Rejection Questionnaire (Rohner and Khaleque 2005)

General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)

All the above measures have already been described in the previous section.

Summary

The assessment protocol is summarised in the following flow chart:


Outline of PGD study plan
Cases:
Identify mothers and prospective children from clinic database
(Identify consecutive births)
Controls:
Identify matched children from Community Child Health database
(Identify consecutive births)
Establish contact via fertility practitioner
Establish contact by direct invitation to participate in the study
Write to families potentially interested with patient information sheets and consent forms (ethically approved)
Arrange family friendly appointments and send out questionnaires
Blinded neurodevelopmental assessments
Blinded collection/completion of questionnaires (for psychosocial/family functioning assessments)
Blinded growth and physical check
Blinded scoring of neurodevelopmental tests and entry into database
Blinded scoring of questionnaires and entry into database
Identify any health concerns and complete the report of health and establish database
Data collected to be analysed
Dissemination
Report findings (via scientific papers/Congresses) and to participating families
Further follow up when children are older?
To inform practice guidelines with other future studies.
Counselling advice for mothers considering pregnancy for RCOG?

Chapter 4

4. Results

4.1 Results of PORD study

4.1.1 Children studied

Out of an original cohort of 150 qualifying children, only 80, living within the London Region were eligible for the study. The other 70 children were not eligible as the child/children were over 8 years or the family had moved and was no longer in London and logistically it was difficult to organise participation. Of the 80 a total of 35 (44%) responded to the invitation and finally 24 (30%) children born between August 1997 and October 2005 were subsequently seen for assessment.

Reasons for non-participation were: some families were unwilling to participate even though they acknowledged the invitation, families had moved abroad, families had moved and were not traceable, families were unwilling to travel in order to be seen, the family did not attend mutually arranged appointments, family declined but the reasons for not doing so were not provided (for ethical reasons it was not possible to ascertain reasons for non participation).

Naturally conceived children were recruited using the local child health database. Children were identified from Community Child Health System which is a community database system with 500,000 children within a group from North London presently involving 9 Primary Care Trusts (PCT). Replies were received from 130 parents of control children. 55 of them were ineligible because of, age and birth order. Children who had emigrated, who were born less than 32 weeks gestation or whose mother tongue was not English were not approached. Finally 37 (49 % participation rate) control children were assessed. Families who had shown interest initially were often unable to attend due to the distance they were required to travel;

the family sometimes didn't attend mutually arranged appointments, mother giving birth to new baby in family, ill health of mother and/or child etc.

4.1.2 Matching

Study children were well matched for age and sex.. There were similar proportions of parents in social classes I, II and III within each conception group.

Table 4.1.1 Matching characteristics of study children:

	PORD	Control	p value
Mean (SD) age at assessment (months)	38.56 (21.31)	44.58 (29.44)	0.36
Sex (male) Frequency (%)	13(50)	16(43)	0.404
Mean gestational age at birth in weeks(SD)	37.04 (4.11)	39.81 (1.77)	0.001

There was no significant difference between the study and control groups in terms of child age at evaluation (Mean PORD = 36.56months, Mean control = 44.58 months; p = 0.995) and the age of evaluation ranged from 8 years 6 months to 3 years 9 months.

4.1.3 Maternal socio-demographic data

There was no difference in the maternal ages between the PORD and the control groups. The mean age of PORD mothers were 37.63 years (SD 6.170) compared to the control mothers' age of 38.97 years (SD 4.324). The groups were not significantly different (t = 1.00; df =59; p = 0.32 NS).

Mothers with renal disease came from a lower socioeconomic background; 7 (29%) mothers from the PORD group were either in social class IV or below compared to only 1 (3%) mother in the control group, who was in social class IV. The difference was statistically significant with $p<0.01$.

Three (13%) mothers from the PORD group had education levels equivalent to A level and below compared to 6 (17%) mothers in the control group. The difference was not statistically significant. Thus control group mothers were as likely to have a university entry level education and an overall higher educational degree compared to PORD mothers.

Mothers from the PORD group were more likely to be married (100%) compared to the control mothers (86%). However the difference was not statistically significant

The number of mothers who smoked was fewer in the PORD group (none) compared to the control group (3%). The difference was not statistically significant.

Current alcohol consumption was less in the mothers in the PORD group compared to the mothers in the control group. The difference was significant with $p<0.05$.

The maternal socio demographic details are summarised in Table 4.1.2

Table 4.1.2 Maternal socio demographic details

	PORD Mothers	Control Mothers	p value
Maternal age in years (SD)	37.04 (4.11)	39.81 (1.77)	0.001
Maternal social class	No. (%)	No. (%)	
1	7 (29)	16 (43)	
2	7 (29)	13 (35)	
3N	2 (8)	7 (19)	
3M	1 (4)	0	
4	2 (8)	1 (2.7)	
5	5 (21)	0	
Maternal education	No. (%)	No. (%)*	
-University entry exam or higher	21 (87.5)	30 (81)	
Subgroups:			
-Higher degree	3 (12.5)	17 (46)	
-Degree	13 (54)	12 (32)	
-University entry	5 (20.8)	1 (2.7)	
-A-level	0	4 (10.8)	
-GCSE/O-level	1 (4)	2 (5)	
-No qualification	2 (8)	0	
Maternal smoking	No. (%)	No. (%)	
-Current smoker	0	1 (3)	0.42
Marital status	No. (%)	No. (%)*	
-married	21 (87.5)	27 (75)	
-single	0	3 (8.3)	
-separated	0	2 (5.6)	
-divorced	0	0	
-living together	3 (12.5)	4 (11)	
-widow	0	0	
Maternal alcohol consumption			
-alcohol units (SD)	1.27 (2.73)	3.47 (4.61)	0.04

*data missing from one control mother

4.1.4 Paternal socio demographic data

There was no significant difference in social class between paternal groups.

Five (23%) of fathers from the PORD group had education levels equivalent to A level and below compared to 3 (9%) fathers from the control group. The difference

was not statistically significant. Thus fathers from both the groups were as likely to have a university entry level education and an overall higher educational degree.

There was no significant difference between the numbers of children that the fathers in each group had from previous relationships.

Current alcohol consumption differed between groups with PORD fathers less likely to drink than the control group of fathers. Current alcohol units in the PORD group was 3.18 (SD 5.04) and in the control group it was 6.16 (SD 6.24). The difference was not statistically significant.

Smoking was more common in the PORD group of fathers when compared with the control group however the result was not statistically significant (PORD 13 % vs. Control 6% p=0.34 NS).

In some cases the information regarding paternal socio demographic details were provided by the mother of the child. This meant that in a few cases, the data was not available. Missing data is indicated in the tables.

The paternal socio demographic details and missing data are summarised in Table 4.1.3.

Table 4.1.3 Paternal socio demographic detail

	PORD fathers (n=24)	Control Fathers (n=37)	p values
Paternal social class	No. (%)	No. (%)	
1	9 (37.5)	18 (49)	
2	7 (29)	13 (35)	
3N	1 (4)	3 (8)	
3M	6 (25)	2 (5.4)	
4	0	0	
5	1 (4)	1 (3)	
Paternal education§	No. (%)	No. (%)	
-University entry exam or higher	17 (77)	31 (91)	
Subgroups:	No. (%)	No. (%)	
-Higher degree	4 (18)	8 (23.5)	
- Degree	9 (41)	18 (53)	
-University entry	4 (18)	5 (21)	
-A-level	4 (18)	2 (6)	
-GCSE/O-level	1 (.5)	1 (3)	
-No qualification	0	0	
Paternal smoking	No. (%)	No. (%)*	
-Current smoker	3 (13)	2 (6)	0.34
Paternal alcohol consumption	No (%)	No. (%)	
-alcohol units (SD)	3.18 (5.04)	6.16 (6.24)	0.06

§ Data missing from renal and control group

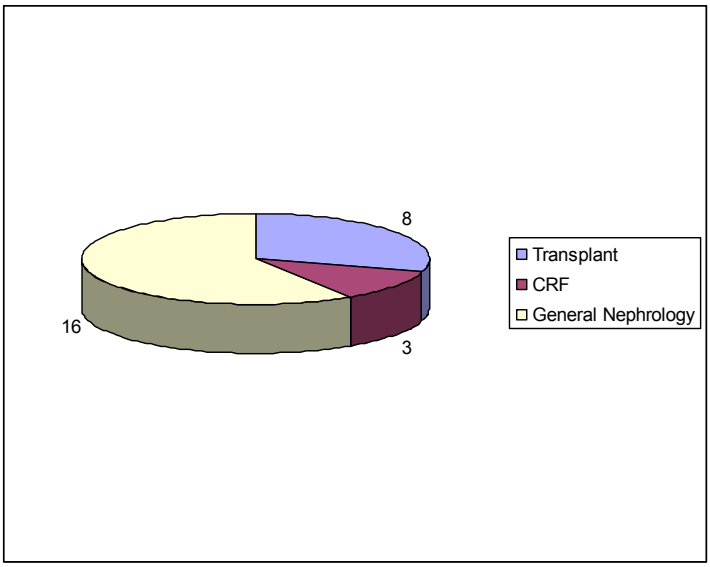
* Data missing from one control father

4.1.5 Maternal Renal Disease

The mothers in this study had a variety of renal disorders. From the renal database of the Royal Free Hospital they were broadly divided in three main categories:

- Transplant
- Chronic renal failure
- General Nephrology

Figure 1 Distribution of maternal renal disease



The general nephrology conditions included various diagnoses: focal segmental glomerular sclerosis, absent kidney (?congenital), single hypertrophied kidney, autoimmune renal disorder, IgA nephropathy, proteinuria, haematuria, raised blood pressure, nephritis, pyeloplasty, reflux nephropathy with nephrectomy and reimplantation of ureters, renal stones –secondary to increased oxalate level. None of the mothers' were pregnant while on dialysis but one mother required dialysis post pregnancy.

The following was the interval between transplant and conception in the group of mothers with renal transplant

Table 4.1.4 Mothers with renal transplant

Cases studied	Interval to 1st Pregnancy (in years)	Interval to 2nd pregnancy (in years)	Further transplant following pregnancy
1	11 from 1st transplant	< 2 from 2nd transplant*	Yes, 8 years from 1st pregnancy
2	5 from 2nd transplant*	-	Yes, 3 years from the 1st pregnancy
3	2	7*	No
4	2	6*	No
5	<2*	-	No
6	8	14*	No
7	12*	15	No
8	12	15*	No

*The actual children who were assessed in the current study.

There were some data available about the renal function of the mothers prior to conception. However a lot of the data was incomplete as it proved very difficult to extract data from the mothers' medical notes. Often notes were missing and not found, notes were often incomplete with missing data and also serial monitoring was not often done.

Women with renal disease also had other medical problems. They were:

hypothyroidism, acoustic neuroma; urinary tract infections, anorexia nervosa, depression, atopic illnesses like asthma, hay fever and eczema, migraine, musculoskeletal problems like back pain, scoliosis (associated with increased frequency of chest infection) and joint problems, epilepsy secondary to tuberous sclerosis, hypertension, pancreatitis and diabetes.

Values of creatinine prior to conception ranged from 63- 176. Haemoglobin levels ranged from 11-15.8. Most mothers had mild proteinuria; severe proteinuria was rare.

4.1.6 Health of PORD mothers in Pregnancy

Illness in pregnancy/obstetric complication:

The number of illnesses/obstetric complications reported by mothers during pregnancy was slightly higher in the PORD group compared to the control group (PORD 46% vs. Control 27%; p= 0.13). The difference was not statistically significant. The PORD mothers reported the following common illnesses: hypertension (n=6), pre eclampsia (n=1), proteinuria (n=3), chest infection (n=1), diabetes (n=1), urinary tract infection (n=3), hyperemesis (n=2). The control group mothers reported the following illnesses: hypertension (n=1), infection (n=1), urinary tract infection (n=1), diabetes (n= 1), musculoskeletal problem (n=5). There were no statistically significant differences in the pattern of illnesses reported in pregnancy by the two groups of mothers.

Medications used:

The numbers of medication used in pregnancy was greater in the PORD group compared with control mothers. The difference was statistically significant with p <0.01. The differences were mainly accounted for by use of immunosuppressant medications the mothers with renal transplant were on. These included azathioprine, cyclosporin, tacrolimus, prednisolone and mycophenolate mofetil. Mothers with renal disease were also on antihypertensive medications, atorvastatin, antibiotics and iron supplement. The control mothers were on medications like insulin, thyroxine, aspirin, antibiotics, iron supplement and anti-D antibodies (short tem use). The use of medications like common analgesics, antacids, antibiotics and vitamins were similar in each group.

Smoking/Alcohol consumption

Smoking in pregnancy was reported in 1 mother from the control group (3%); none of the PORD mothers smoked in pregnancy. There was no difference noted between maternal groups for alcohol consumption in pregnancy. None of the mothers in either group were reported to consume alcohol in pregnancy.

Support in pregnancy

The number of adults available to support mothers during pregnancy was higher in the control group compared to the PORD group mothers (PORD 83% vs. control 100%; p= 0.01). The difference was statistically significant p<0.01.

Breast feeding:

Intention to breast feed was significantly higher in the control group with 33 (94%) of mothers wanting to breast feed compared with 15 (65%) of the renal mothers wanting to breast feed. This was significantly different p<0.01. However a lot of these mothers were precluded from breast feeding because of the medications they were on and were often strongly advised against breast feeding.

Type of labour

The PORD mothers were less likely to have spontaneous onset of labour when compared with the control group of mothers (PORD 60% vs. Control 79%, p=0.14). The difference was not statistically significant. Incidence of caesarean sections, planned and emergency was higher in the PORD group. There was also a significant difference in instrumental deliveries with 5 (20.8%) of PORD mothers having instrumental deliveries compared with 2 (5.4%) of control mothers. Control mothers were most likely to have normal vaginal delivery and significantly fewer vaginal deliveries were reported for the cases.

There was some missing data for perinatal results as indicated in the tables. The numbers involved are small, often missing data from a single child. This missing information is often due to parental difficulty recalling specific information.

Health of PORD mothers in pregnancy are summarised in tables 4.1.5; 4.1.6 and 4.1.7

Table 4.1.5 Details of health of mothers in pregnancy

	PORD mothers (n=24)	Control group (n=37)	p value
	No. (%)	No. (%)	
Illness in pregnancy	11 (46)	10 (27)	0.13
Medications taken during pregnancy	17 (71)	7 (19)	0.001
Alcohol consumption in pregnancy - current drinker	0	0	
Smoking in pregnancy	0	1 (3)	
Intention to breast feed* - Yes - No	 15 (65) 8 (35)	 33 (94) 2 (6)	0.001
Adult support for mother§ - yes - No	 20 (83) 4 (17)	 37 (100) 0	0.01
Labour - spontaneous - induced - no labour	 12 (50) 8 (33) 4 (17)	 26 (70) 7 (19) 4 (11)	0.14
Method of delivery ψ - vaginal - Forceps/ventouse - Planned caesarean - Emergency caesarean	 9 (37.5) 5 (20.8) 4 (16.7) 6 (25)	 27 (73) 2 (5.4) 4 (10.8) 4 (10.8)	0.04
Gestational age at birth – weeks Mean (SD)	37.04 (4.11)	39.81 (1.77)	0.001

*Missing data from one PORD mothers & 2 control mothers
§Missing data from 3 control mother
ψ There was a significant association between group and mode of delivery (X^2 = 8.29, df= 3, p= 0.04)

Table 4.1.6 Illness in pregnancy/obstetric complications

	PORD mothers	Control group
Hypertension	6	1
Pre eclampsia	1	0
Proteinuria	3	0
Infection	1	1
Hyperemesis	2	0
Diabetes	1	1
Urinary tract infection	3	1
Musculoskeletal problems	0	5

Table 4.1.7 Medications in pregnancy

	PORD mothers	Control group
Antibiotics	2	1
Antihypertensive	4	0
Azathioprine	4	0
Cyclosporin	4	0
Tacrolimus	3	0
Mycophenolate mofetil	2	0
Steroids (Prednisolone)	6	0
Heparin	0	1
Insulin	0	2
Thyroxine	0	3
Iron	1	1
Atorvastatin	2	0
Others	2	3

4.1.7 Neonatal Results

There was significant difference in the distribution of gestational age in the two groups studied. The PORD group had a significant lower gestational age (gestational age for PORD 37.04 (S.D 4.11) vs. control 39.81(S.D 1.77)). This was statistically significant $p<0.01$.

The PORD group also had lower birth weight, and higher number with birth weight less than 2500g (birth weight (gms) PORD 2842.57 (S.D 886) vs. control 3414.07 (S.D 488); $p <0.05$.

PORD children were more likely to require resuscitation after birth when compared to the control group; PORD 6(25%) vs. control 3 (8%); the result did not reach statistical significance.

The number of babies admitted to the neonatal unit was different in the two groups. 8 babies (33%) born after PORD were admitted to the neonatal unit compared to 3 babies in the control group (8%). This difference was clinically significant $p < 0.05$. The duration of stay in the neonatal unit differed in the two groups. In the PORD group 6 (25%) babies spent > 7 days in the neonatal unit compared to 2 (5%) babies from the control group, $p = 0.782$. There were inadequate numbers in the two groups to compare the significance of stay in neonatal unit in terms of length of stay ≥ 7 days.

Ventilation occurred more commonly in the PORD group though this difference was not statistically significant. In the PORD group 4 (17%) babies got ventilated compared to 1 (3%) in the control group, $p= 0.052$. Two of the babies in the PORD group were ventilated for 1 and 2 days respectively because of prematurity. The ventilatory support required was minimal. The other two babies were ventilated for 15 and 30 days. These two babies were premature and had respiratory distress and sepsis. The baby in the control group who was ventilated was delivered prematurely at 31 weeks by emergency section secondary to maternal placental abruption and was only ventilated for half a day.

Reasons for admission to the neonatal unit included a range of common neonatal problems. There was a difference seen between groups, with more control babies (10 (27.5%) with reported neonatal illnesses when compared with the PORD group 3 (12.5%). The types of illnesses that affected the children as neonates also

varied. Some of these illnesses did not necessarily require admission to a neonatal unit.

Mothers from the PORD group were less likely to exclusively breastfeed their babies than mothers from the control group. Nine (39%) mothers from the PORD group did not exclusively breast feed for at least one month compared to 4 (11%) mothers in the control group, this was statistically significant $p < 0.01$. Of the mothers who chose to breast feed, the length of exclusive breast feeding was similar between groups.

Table 4.1.8 Neonatal details

	PORD (n= 24) Frequency (%)		Control (n= 37) Frequency (%)		p value
Male	13 (50)		16 (43)		0.404
Birth weight Mean (SD)	2842 .6 (886)		3414.0 (488)		0.002
Resuscitation Yes/No	Yes	6 (25)	Yes	3 (8)	0.070
	No	18 (75))	No	34 (92)	
Admission to NNU Yes/No	Yes	8 (33)	Yes	3 (8)	0.012
	No	16 (67)	No	34 (92)	
Length of stay on NNU Not admitted 1-7 days > 7 days	16 (67) 2 (8) 6 (25)		34 (92) 1 (3) 2 (5)		
Ventilation required	Yes	4 (17)	Yes	1 (3)	0.052
	No	20 (83)	No	36 (97)	
Neonatal illness	Yes	3 (12.5)	Yes	10 (27.5)	
	No	21 (87.5)	No	27 (73)	
Type of feeding -exclusive breast -mixed -formula	14 (61)* 3 (13) 6 (26)		32 (86.5)* 2 (5.5) 2(5.5)		0.011
Length of exclusive breast feeding -not applicable <4weeks -5-8 weeks -9-12 weeks >12 weeks	18 3 1 4 23		29 3 2 4 26		
Adult support for mother -Yes -No	47 (96) 2 (4)		52 (78.8) 14 (21)		

* Missing data from both groups

4.1.8 Physical assessment

Hospital admissions were similar in the PORD group compared to the control group (PORD 2(8%) vs. Control 3(8%) p = 0.975). Number of illnesses reported by parents were more common in the control group compared to the PORD group (PORD 7 (29%) vs. Control 29 (78%): p = 0.000). Illnesses were classified into respiratory tract infections, common childhood infections, fractured bones, gastrointestinal, atopic (eczema, asthma) and other illnesses. Of these, there was a trend towards control group children having atopic illnesses, chicken pox, common childhood infections and fractured bones more commonly than the PORD group.

Childhood medication use was similar between the groups.

There was no difference between the two groups regarding surgery. General examination of both groups did not reveal any abnormalities apart from three control children with undescended testicle, tight foreskin (which was asymptomatic) and eczema respectively. Two children from the PORD group had mild eczema.

4.1.9 Growth

There were no differences noted between groups for childhood growth in terms of actual height in cms and weight in kgs and centiles. These centiles were calculated accurately using the L-grow® computer programme standardised for the UK population of children (LGROW 1998). However there was no software to calculate head circumference centiles. The head circumference data was therefore based on raw measurement only, but no significant difference between head circumference were seen.

Table 4.1.9 .Physical development at time of evaluation

		Case	Control	p
Height	N	24	33	
	Mean	99.55	97.72	0.69
	SD	21.34	13.79	
Weight	N	24	35	
	Mean	16.98	15.34	0.29
	SD	7.67	4.23	
OFC	N	23	34	
	Mean	50.30	50.14	0.85
	SD	3.77	2.60	

OFC= Head circumference

4.1.10 Congenital malformation

There was no difference between incidences of congenital malformations, major or minor in the PORD and the control groups.

4.1.11 Griffiths Mental Developmental Scales

Data was available from 56 (21 cases and 35 controls) children for the Griffiths Mental development Scales. Two children assessed in the PORD group were above 7 years 11month (cut off for eligibility of Griffiths). However both these children were progressing without any concern and school reports were above average which suggested normal development. One child in the PORD group scored well below the accepted norms. Two control children did not complete the assessment due to lack of cooperation and so could not be included in this assessment.

The data indicates that there was little difference between the groups. The mean Griffiths quotient was 106.29 (SD 18.48) for the PORD group and 105.65(SD 11.60) for the control group which were both within the normal range, and did not differ significantly ($p = 0.87$).

Table 4.1.10 Group differences on the overall and subscale score on the Griffiths mental development scales.

Griffiths Scales		Case	Control	p
Locomotor subscale	N	21	35	
	Mean	108.84	108.91	0.98
	SD	14.77	17.23	
Personal social subscale	N	21	35	
	Mean	105.04	106.77	0.69
	SD	19.16	13.79	
Hearing speech subscale	N	21	35	
	Mean	107.66	109.60	0.73
	SD	26.55	16.15	
Eye hand subscale	N	21	35	
	Mean	101.02	98.57	0.59
	SD	18.37	15.58	
Performance subscale	N	22	35	
	Mean	107.60	106.06	0.74
	SD	25.90	14.50	
Practical reasoning subscale	N	15	21	
	Mean	103.01	112.12	0.37
	SD	43.33	13.31	
Griffiths General Quotient	N	21	35	
	Mean	106.29	105.65	0.87
	SD	18.48	11.60	

4.1.12 Child and Family Relationships

Parental questionnaires were used to assess family functioning.

Parents were posted the questionnaires once an appointment date had been fixed. They were sent out at least a week ago with the details of the appointment. The parents were requested to complete the questionnaires before the assessment as much as possible. Any queries related to the questionnaires or the completion was addressed during the actual assessment. They were requested to bring the questionnaires with them for the appointment.

The set included the following questionnaires:

1. General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)
2. The Parental Acceptance and Rejection Questionnaire (Rohner and Khaleque 2005)
3. The parenting Stress Index /Short form (Abidin 1990)
4. The Carey Temperament Scales (Second Edition): of which the following were used:
    a. Early Infancy Temperament Questionnaire for 1 to 4 month old infants.
    b. Revised Infant Temperament Questionnaire for 4 to 11 month old infants.
    c. Toddler Temperament Scale for 1-2 year old children.

5. Child Behaviour Checklist for ages 1½-5 and 6-18

Response rate:

Overall there were 4 sets of missing questionnaires in the PORD group. This meant an 83 % response rate. Mothers of two children did not return any of the set. This family even though English speaking was actually from Albania and possibly had practical difficulties in completing the questionnaires. It was not possible to contact the family once the assessment was complete to offer help in completing the questionnaires. The other two had 1-3 of the questionnaires missing from the set. A second copy of the set or missing questionnaires were sent by post. A further telephone reminder was attempted where possible. Thereafter it was taken as a non-response.

a. Child Behaviour Check List (CBCL)

Maternal reports of the child behavioural trends were obtained from 16 children from the PORD group and 20 children from the control group. The CBCL 1½- 5 and 6-18 can be scored in terms of two broad groupings of syndromes designated as *Internalising and Externalising. Other problems* (which were obtained from open ended question where a parent can list up to three problems which were not listed in the actual questionnaire) were also scored and the summative value of the Internalising, Externalising and the Other problems score gave us the total problem scales. The Internalizing grouping reflects problems within the self such as anxiety, depression, somatic complaints without known medical cause and withdrawal from social contacts. The Externalising grouping represents conflicts with other people and their expectations for the child's behaviour such as rule breaking and aggressive behaviour.

The total sores of Internalising were not significantly different in the two groups, however the T scores were slightly different though not significant ($p= 0.087$). The total and the T scores for Externalising were significantly different between the two groups ($p< 0.05$ and $p<0.01$). The scores for other problems were also significantly different in the PORD and control group ($p<0.01$). The total problem score and the total problem T score were significantly different in the two groups ($p<0.05$)

The T score is a particular kind of standard score designed to provide a similar quantitative metric for scales that have different numbers of items and different distributions of scores

Table 4.1.11 Group differences on the overall and subscale score on the Child Behaviour Check List

	Group		
	Case Mean (SD)	Control Mean (SD)	p
Internal 1	7.81 (5.45)	5.15 (3.57)	0.087
Internal 2	50.93 (8.56)	45.25 (8.24)	0.051
External 1	13.31 (7.96)	7.35 (6.28)	0.017
External 2	53.06 (8.91)	43.60 (10.73)	0.008
Other Problems	11.72 (4.26)	5.05 (3.36)	0.001
CBCL Total 1	33.81 (18.60)	19.90 (11.80)	0.010
CBCL Total 2	52.12 (9.46)	43.80 (9.07)	0.011

b. Carey Temperament Scales

There was no difference in the temperamental characteristics perceived by mothers in both the group. The questionnaire is a measure of nine NYLS (New York Longitudinal Study) temperament characteristics: activity level, rhythmicity, approach-withdrawal, adaptability, intensity, mood, attention span and persistence, distractibility and sensory threshold.

Table 4.1.12 Group differences on the overall and subscale scores on the Toddler Temperament test.

		Case	Control	p
Activity	N	5	16	
	Mean	4.43	4.08	0.182
	SD	.13	.54	
Rhythmicity	N	5	16	
	Mean	2.49	2.79	0.52
	SD	1.052	.89	
Approach	N	5	16	
	Mean	3.62	3.04	0.12
	SD	.98	.60	
Adaptability	N	5	16	
	Mean	3.40	2.92	0.07
	SD	.79	.40	
Intensity	N	5	16	
	Mean	4.06	3.80	0.56
	SD	.91	.81	
Mood	N	5	16	
	Mean	3.31	2.82	0.18
	SD	1.035	.55	
Persistence	N	5	16	
	Mean	3.30	3.19	0.82
	SD	1.26	.82	
Distractibility	N	5	16	
	Mean	3.56	3.8	0.63
	SD	1.09	.90	
Threshold	N	5	16	
	Mean	4.49	4.34	0.72
	SD	.50	.85	

c. Parenting stress Index (PSI)

There was no difference between the reported parental distresses in the two groups.

Table 4.1.13 Group differences on the overall and subscale score on the PSI test.

		Case	Control	p
PSI parental distress	N	21	37	
	Mean	26.48	26.65	0.94
	SD	9.527	8.593	
PSI parent child dysfunctional interaction	N	21	37	
	Mean	18.76	17.22	0.41
	SD	9.099	5.350	
PSI difficult child	N	20	37	
	Mean	28.45	25.16	0.11
	SD	7.605	7.377	
PSI total clinical score	N	20	37	
	Mean	73.80	69.03	0.36
	SD	20.707	17.320	

d. Parental Acceptance and Rejection Questionnaire (PARQ)

There was no difference in the overall and subscale scores of the Parental acceptance and rejection between the PORD and the control groups.

Table 4.1.14 Group differences on the overall and subscale scores on the PARQ test.

		Case	Control	p
PARQ warmth affection	N	19	37	
	Mean	23.16	24.22	0.39
	SD	3.640	4.650	
PARQ aggression hostility	N	19	37	
	Mean	25.11	27.35	0.29
	SD	7.031	7.754	
PARQ neglect indifference	N	19	37	
	Mean	21.00	20.43	0.65
	SD	5.077	4.207	
PARQ rejection	N	19	37	
	Mean	15.53	14.73	0.56
	SD	5.501	4.568	
PARQ total	N	19	37	
	Mean	84.79	86.73	0.70
	SD	18.229	17.508	

e. General Health Questionnaire (GHQ-28)

No differences were identified between the two groups using the GHQ-28, which was designed to detect current mental health problems in the general population. The GHQ-28 provides four dimensions, measuring somatic symptoms, anxiety and insomnia, social dysfunction and severe depression.

Table 4.1.15 Group differences on the subscale and total score on the GHQ

General Health Questionnaire	Case Mean (SD)	Control Mean (SD)	p value
GHQ somatic symptoms	1.09 (4.78)	1.59 (2.39)	0.59
GHQ anxiety and insomnia	1.80 (2.24)	1.64 (2.40)	0.82
GHQ social dysfunction	0.47 (0.81)	1.18 (1.89)	0.10
GHQ severe depression	0.28 (0.71)	0.35 (.94)	0.78
GHQ Total score	4.57 (4.95)	4.75 (6.49)	0.91

4.2 Results of the PGD study

4.2.1 Children studied

A combined estimate from the four PGD centres suggested that at least 100 children less than eight years could be recruited for the study. Out of an original cohort of 100 qualifying children (children below the ages of 8 years who were born following Preimplantation Genetic Screening or Diagnosis were eligible to participate), only 70 living within the London Region were eligible for the study. The other 30 children were not invited to participate because families had come to London solely for PGD from countries like Saudi Arabia, Egypt, or the United Arab Emirates, where the main language of the children wasn't English and the practicalities of participation were felt to be difficult. Of these 70 a total of 49 (70% participation rate) born between October 2002 and January 2007 were subsequently seen for assessment. This included 4 pairs of twins and 41 singletons. Out of the total 49 children assessed, 39 children were born after PGD for aneuploidy screening (preimplantation genetic screening) and 10 children for known genetic disorders in their families. Of these 10 families, 4 families had chromosomal translocation, 3 families had Down syndrome occur in a previous pregnancy and 3 families had a family history of myotonic dystrophy. Refer to figure 4.2 and 4.3:

Figure 2 Distribution of PGD children studied

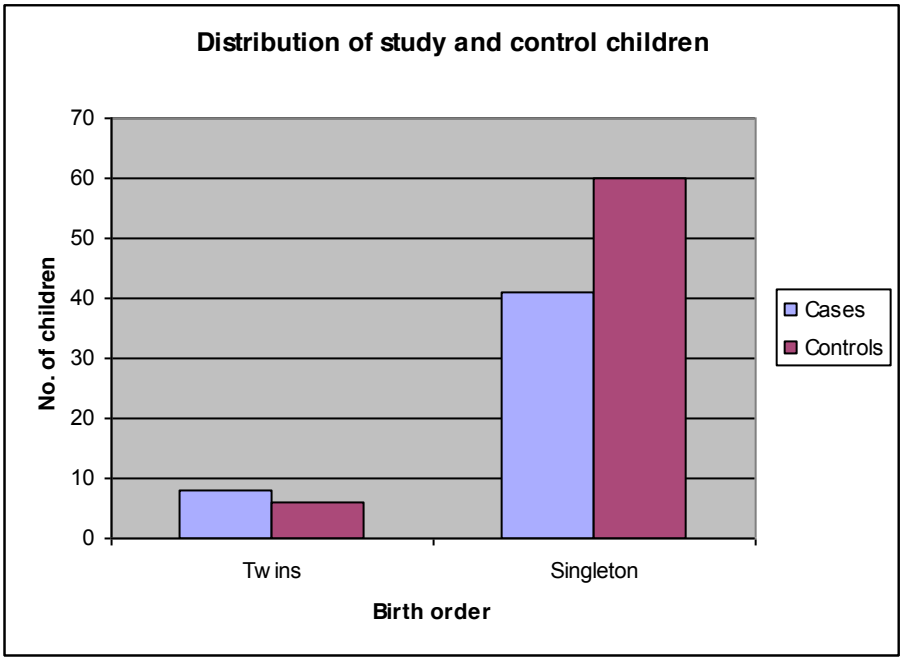
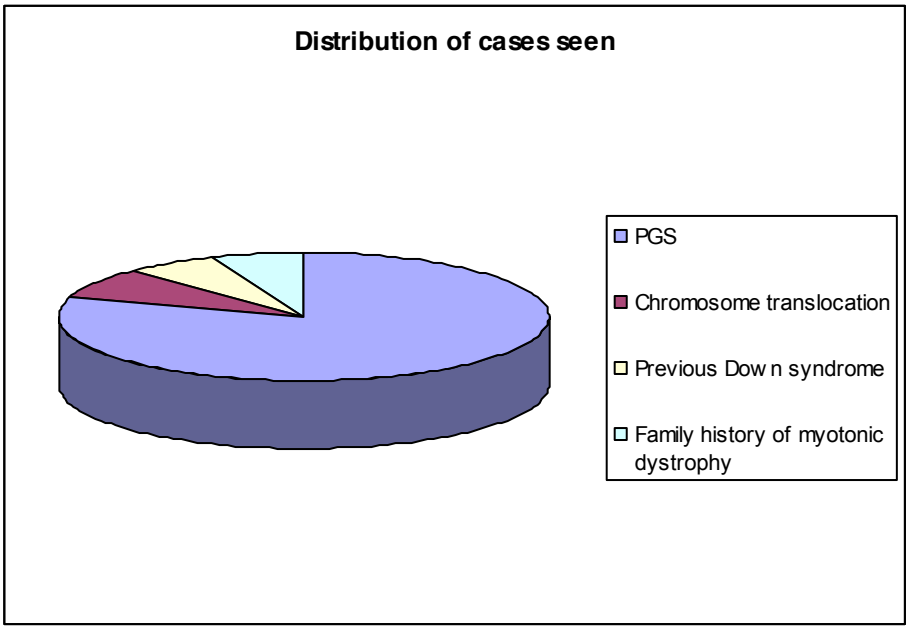


Figure 3 PGD cases seen



Reasons for non-participation were: families had moved abroad, families had moved and were not traceable, families were unwilling to travel in order to be seen, the family did not attend arranged appointments, family declined but the reasons for not doing so were not provided (for ethical reasons it was not possible to ascertain reasons for non participation).

Naturally conceived children were recruited from local nurseries for the control group. The London based Multiple Birth Foundation was approached for recruitment of twin controls. Replies were received from 175 parents of control children;. 85 of them were ineligible because of age and birth order. Children who had emigrated, who were born less than 32 weeks gestation or whose mother tongue was not English were not approached. Finally 66 (73 % participation rate) control children were assessed (3 pairs of twins and 60 singletons). Families who had shown initial interest were often unable to attend due to the distance they were required to travel; the family sometimes didn't attend mutually arranged appointments, mother giving birth to new baby in family, ill health of mother and/or child etc.

4.2.2 Matching

Study children were well matched for age, sex, gestational maturity. There were similar total number of parents in social classes I, II and III within each conception group.

Table 4.2.1 Matching characteristics of study children:

	PGD	Control
Mean (SD) age at assessment (months)	18.38 (11.43)	18.29 (5.82)
Boys (%)	28 (57)	32 (49)
Gestational age (SD) at birth (weeks)	38.24 (2.58)	40.03 (1.40)

There was no significant difference between the study and control groups in terms of infant age at evaluation (Mean PGD = 18.4 months, Mean control = 18.3 months; t = -0.056, df =114, p = 0.995) and the age of evaluation ranged from 3 months to 56 months.

4.2.3 Maternal socio-demographic data

The PGD mothers were significantly older than the natural conception group. The mean age of PGD mothers were 40.40 years (3.74) compared to the control

mothers age of 35.39 years (4.30). This is not surprising in view of the history of infertility in these women who underwent PGD for aneuploidy screening (PGS).

There was no significant difference in social class between maternal groups. Control group mothers were as likely to have a university entry level education and an overall higher educational degree compared to PGD mothers. Only 9 mothers from the PGD group and 8 mothers in the control had education levels below university entry, the difference was not significant ($p = 0.375$).

Mothers from the PGD group were more likely to be married (81.6%) compared to the control mothers (75.7%)

The number mothers who smoked were fewer in the PGD group (none) compared to the control group (9%).

Current alcohol consumption was less in the mothers in the PGD group compared to the mothers in the control group (PGD 55% vs. Control 78.8%).

The maternal socio demographic details are summarised in Table 4.2.2

Table 4.2.2 Maternal socio demographic details

	PGD Mothers n = 49	Natural conception Control Mothers n = 66
Maternal age in years (SD)	40.4 (3.74)	35.39 (4.30)
Maternal social class[§]	No. (%)	No. (%)
1	30 (61)	20 (30)
2	8 (16)	23 (34)
3N	11 (23)	18 (27)
3M	0	0
4	0	3 (5)*
Maternal education	No. (%)	No. (%)
-University entry exam or higher	40 (82)	58 (88)
Subgroups:		
-Higher degree	23 (47)	33 (50)
-Degree	13 (27)	19 (29)
-University entry	4 (8)	6 (9)
-A-level	9 (19)	7 (11)
-GCSE/O-level	0	1 (2)
-No qualification	0	0
Maternal smoking	No. (%)	No. (%)
-Current smoker	0	6 (9)
Marital status	No. (%)	No. (%)
-married	40 (82)	50 (76)
-single	2(4)	2 (3)
-separated	0	0
-divorced	0	0
-living together	7 (14)	14* (21)
-widow	0	0
Maternal alcohol consumption	No (%)	No. (%)
-current drinker	27 (55)	52 (79)

*Includes one couple in civil partnership

[§]Includes missing data.

4.2.4 Paternal socio demographic data

Fathers of PGD children were older than fathers of control children (mean age PGD: 41.26 vs. Control: 38.46; p = 0.076), though the difference was not significant. There were no significant differences in social class between paternal groups. Control group fathers were as likely to have a university entry level education and an overall higher educational degree compared to PGD group fathers (PGD 83.6% vs. Control 86.3% p = 0.721, not significant).

There was no significant difference between the numbers of children that the fathers in each group had from previous relationships. However, there was a trend towards PGD fathers having two or more children from previous relationships.

Current alcohol consumption differed between groups with PGD fathers possibly being more likely to drink than the control group of fathers (PGD 79.6% vs. Control 74.24%%; chi-square =.448, df =1, p= 0.503), though the difference was not significant. Smoking seemed less common in the PGD group of fathers when compared with the control group, although again this was not statistically significant (PGD 6.1% vs. Control 16.6%; chi-square=3.35, df =1, p= 0.067).

In some cases the information regarding paternal socio demographic details were provided by the mother of the child. This meant that in a few cases, the data was not available.

The paternal socio-demographic details and missing data are summarised in Table 4.2.3.

Table 4.2.3 Paternal socio demographic detail

	PGD fathers (n=49)	Natural conception Control Fathers (n=66)
Paternal age in years (SD)	41.26 (5.37)	38.46 (10.02)
Paternal social class	No. (%)	No. (%)
1	36 (74)	26 (39)
2	9 (18)	21 (32)
3N	4 (8)	8 (12)
3M	0	8 (12)
4	0	3 (5)
Paternal education	No. (%)	No. (%)
-University entry exam or higher	41 (84)	57 (86)
Subgroups:	No. (%)	No. (%)
-Higher degree	25 (51)	30 (46)
-Degree	9 (18)	21 (32)
-University entry	7 (14)	6 (9)
-A-level	7 (14)	8 (12)
-GCSE/O-level	1(2)	1 (1)
-No qualification		
Paternal smoking	No. (%)	No. (%)*
-Current smoker	3 (6)	11 (17)
Paternal alcohol consumption	No (%)	No. (%)
-current drinker	39 (80)	49 (74)

* Data missing from one control father

4.2.5 Health of PGD mothers in Pregnancy

Illness in pregnancy/obstetric complications:

The number of illnesses/obstetric complications reported by mothers in pregnancy was higher in the PGD group compared to the control group (PGD 40% vs. Control 21%; chi-square = 5.19, df = 1, p= 0.022). The PGD mothers reported the following common illnesses: chest infection (n=2), diabetes (n=3), bleeding in pregnancy, urinary tract infection (n=3), hypertension, gastrointestinal symptoms (n=2), anaemia (n=2), hyperemesis (n=4). The control group mothers reported the following illnesses: hypertension (n=2), intra uterine growth retardation, bleeding in pregnancy, cardiac arrhythmias (Wolff-Parkinson-White syndrome), chest infection (n=4), gastrointestinal symptoms (n=2) and septicaemia. There were no

statistically significant differences in the pattern of illnesses which were reported in pregnancy by both groups of mothers.

Medications used:

The number of medications used in pregnancy was greater in the PGD group compared with control mothers (PGD 73.5% vs. control 32.3%; chi-square=19.51, df =1, p= 0.00). The differences were mainly accounted for by use of progesterone, aspirin, steroids (hydrocortisone and prednisolone), heparin and Immunoglobulins. These medications were an integral part of the IVF process. The use of medications like common analgesics, antacids, antibiotics and vitamins were similar in each group.

Smoking /Alcohol consumption:

Smoking in pregnancy was reported in 3 mothers from the control group (4.5%); none of the PGD mothers smoked in pregnancy. There was a significant difference noted between maternal groups for alcohol consumption in pregnancy. None of the PGD mothers were reported to consume alcohol in pregnancy compared to 11 (16.6%) mothers in the control group who were reported to drink between 1-10 units of alcohol in pregnancy. The study did not specify when in the pregnancy or 'for how long' the mothers consumed alcohol.

Support in pregnancy:

The number of adults available to support mothers during pregnancy was also higher in the PGD group compared to the control group of mothers (PGD 93.8% vs. Control 77.7%; chi-square = 0.194, df =1, p = 0.659). Intention to breast feed was similar between groups at the time of pregnancy (PGD 91% vs. Control 89.4%).

Type of labour:

The PGD mothers were less likely to have spontaneous onset of labour when compared with the control group of mothers (PGD 40.8% vs. Control 60.6 %, chi-square=4.414, df =1, p= 0.035). This difference was significant. Incidence of Caesarean sections, planned and emergency was higher in the PGD group. There

was no significant difference in the occurrence of instrumental delivery. Control mothers were most likely to have a normal vaginal delivery.

There is some missing data for perinatal results as indicated in the tables. The numbers involved are small, often missing data from a single child. This missing information is often due to parental difficulty recalling specific information.

Health of PGD mothers in pregnancy are summarised in tables 4.2.4; 4.2.5 and 4.2.6

Table 4.2.4 Details of health of mothers in pregnancy

	PGD mothers (n=49)	Naturally conceived control group (n=66)
	No. (%)	No. (%)
Illness in pregnancy	20 (40)	14 (21)
Medications taken during pregnancy	36 (74)	21 (32)
Alcohol consumption in pregnancy - current drinker	0	11 (16.6)
Smoking in pregnancy	0	3 (4.5)
Intention to breast feed* - Yes - No	 45 (92) 2 (4)	 59 (90) 3 (5)
Adult support for mother§ - yes - No	 46 (94) 3 (6)	 49 (78) 14 (22)
Labour - spontaneous - induced - no labour	 20 (41) 11 (22) 18 (37)	 40 (61) 16 (24) 10 (15)
Method of delivery - vaginal - Forceps/ventouse - Planned caesarean - Emergency caesarean	 13 (27) 5 (10) 17 (35) 14 (21)	 35 (53) 12 (18) 7 (11) 12 (18)
Gestational age at birth – weeks Mean (SD)	38.24 (2.58)	40.03 (1.40)

*Missing data from two PGD mothers & 4 control mothers

§Missing data from 3 control mothers

Table 4.2.5 Illness in pregnancy/obstetric complications

	PGD mothers	Naturally conceived control group
Pregnancy induced hypertension	1	2
Bleeding during pregnancy	1	1
Infection during pregnancy	5	5
Gastrointestinal illness	2	2
Anaemia	2	0
Hyperemesis	4	0
Gestational diabetes	3	0
Any illness in pregnancy	20	14

Table 4.2.6 Medications in pregnancy

	PGD mothers	Naturally conceived control group
Antibiotics	3	4
Asthmatic drugs	0	2
Aspirin	13	1
Progesterone	10	1
Vitamins/Electrolyte	0	7
Iron	0	2
Antihypertensive medication	0	1
Steroids	21	1
Heparin	20	0
Immunoglobulins	12	0
GI medicine	4	1
Insulin	2	0
Thyroxine	2	1
Salbutamol	0	2
Any medication	36	21

4.2.6 Neonatal Results

There was a significant difference in the distribution of gestational age in the two groups studied. The PGD group had a significantly lower gestational age and a higher number with gestation age less than 37 weeks (Gestational age for PGD 38.24 (S.D 2.58) vs. control 40.03 (S.D 1.40); $p = 0.000$) and gestation < 37 weeks (PGD 7 (14%) vs. control 1 (1.5%); $p = 0.009$). The PGD group also had lower birth weight, and a higher number with birth weight less than 2500g (birth

weight (gms) PGD 3122 (S.D 915) vs. control 3440 (S.D 453); p = 0.030 and birth weight < 2500 gms PGD 12 (24.5%) vs. control 1 (1.5%); p = 0.000).

PGD children were not more likely to require resuscitation after birth when compared to the control group (PGD 4 (8%) vs. control 6 (9%), p = 0.836). The proportion of babies admitted to a neonatal unit was slightly different in the two groups: 8 babies (16%) born after PGD were admitted to the neonatal unit compared to 7 babies in the control group (10.6%). This difference was not statistically significant p= 0.391. The number of days spent in the neonatal unit did not differ between the two groups. There were inadequate numbers in the two groups to compare the significance of stay in a neonatal unit in terms of length of stay ≥ 7 days. Mechanical ventilation occurred more commonly in the control group; however the proportion was not significant. Two babies (4%) born after PGD were ventilated. One was ventilated for 4 days because of septicaemia and meningitis and the second was ventilated because of meconium aspiration and the duration could not be recalled by parents. Three babies in the control group were ventilated, one for transient tachypnoea of the newborn (TTN) (duration not recalled), one for meconium aspiration for 5hrs and the last could not recall the duration or the reason for ventilation. Reasons for admission to a neonatal unit included a range of common neonatal problems. There was no difference seen between the groups. The types of illnesses that affected the children as neonates also varied. These illnesses did not necessarily require admission to the neonatal unit.

Mothers from the PGD group were more like to exclusively breastfeed their babies than mothers from the control group (PGD 63% vs. control 53%; chi-square=1.20, df =1, p=0.272). Of the mothers who chose to breast feed, the length of exclusive breast-feeding was similar between the groups.

Table 4.2.7 Neonatal details

	PGD (n = 49)		Naturally conceived control (n = 66)	
Male (%)	28 (57)		32 (49)	
Resuscitation No. (%)	Yes	4 (8)	Yes	6 (9)
	No	45 (92)	No	60 (91)
Admission to NNU No (%)	Yes	8 (16)	Yes	7 (10)
	No	41 (84)	No	59 (90)
Length of stay in NNU -not admitted <1 day -1-7 days >7 days	41 1 4 3		59 0 8 1	
Ventilation required No. (%)	Yes	2 (4)	Yes	3 (4.5)
	No	47 (96)	No	63 (95.5)
Congenital anomalies	2 (4)		3 (4.5)	
Type of breast feeding -exclusive breast -mixed -formula	31 (63) 15 (31) 3 (6)		35 (53)* 20 (30) 9 (14)	
Length of exclusive breast feeding -not applicable <4 weeks -5-8 weeks -9-12 weeks >12 weeks	18 3 1 4 23		29 3 2 4 26	
Adult support for mother -Yes -No	47 (96) 2(4)		52 (79) 14 (21)	

NNU: Neonatal unit

*2 missing data from control group

4.2.7 Physical assessment

Hospital admissions were marginally less common in the PGD group compared to the control group (PGD 4(8%) vs. control 9(14%); chi-square =.03, df =1, p = 0.862). Number of illnesses reported by parents were also similar in each group (PGD 25(51%) vs. control 31(47%): chi-square=.18, df = 1, p = 0.667). Illnesses were classified into upper respiratory tract, lower respiratory tract, dermatological, gastro-intestinal, atopic and other illnesses. Of these, there was trend towards less illness in the control group for gastrointestinal and atopic complaints.

Childhood medication usage was similar between the groups.

There was no difference between the two groups regarding surgery (PGD 2 (4%) vs. Control 3 (5%) chi-square =.01, df =1, p = 0.902). The children in the PGD group underwent minor surgical procedures such as circumcision and pyloromyotomy. The control group required minor plastic surgical procedures and corrective surgery for club foot.

General examination of both the groups did not reveal any abnormalities apart from a single control child with hypospadias.

4.2.8 Growth

There were no differences noted between groups for childhood growth in terms of actual height in cms and weight in kgs and centiles. These centiles were calculated accurately using the L-grow® computer programme standardised for the UK population of children (LGROW 1998). However there was no software to calculate head circumference centiles. The head circumference data was therefore based on raw measurement only, but no significant difference between head circumference were seen.

Table 4.2.8 Physical characteristics at time of evaluation.

	PGD	Natural Conception	p
Age at evaluation months (SD)	18.38 (11.43)	18.29 (5.82)	0.959
Height (SD)	77.50 (17.06)	81.14 (6.78)	0.136
Weight (SD)	11.27 (3.31)	11.75 (1.97)	0.356
Head (SD)	47.75 (6.43)	47.77 (2.06)	0.975

There was no significant difference between the groups in terms of infant age at evaluation (t = -0.056, df =114, p =0.959). A one-way multivariate analysis of variance (MANOVA) was conducted using the two groups as the independent variable and height, weight and head circumference as the dependent variables. The omnibus test was non-significant (F (3,100) = 0.857, p = 0.466) and all tests of between–groups effects were non-significant. A multivariate analysis of co-variance (MANCOVA) was conducted with age at evaluation entered as a covariate and the omnibus test was non-significant (F (3,99) = 2.235, p = 0.089).

4.2.9 Congenital malformation

There was no difference between incidence of congenital malformations, major or minor in the PGD and the control groups. Two children (4%) from the PGD group had reported two minor malformations (strawberry naevus and abnormality to the shape of the pinna) and 3 children (4.5%) from the control group reported two major (Duane's syndrome, a congenital malfunction of eye muscles and hypospadias) and one minor (plagiocephaly). Some of the children in the PGD and the control groups would classify as having major anomalies according to definitions used in previous studies (Bonduelle *et al.*. 2005). However, in this study we defined a major malformation as one in which surgery or ongoing medical monitoring was needed.

4.2.10 Griffiths Mental Developmental Scales

Data was available from 114 (49 cases and 65 controls) children for the Griffiths Mental development Scales. One control child did not complete the assessment due to lack of cooperation and so could not be included in this analysis.
The data indicated that there was little difference between the groups. The mean Griffiths quotient was 102.7 (+/- 13.1) for the PGD group and 103.3 (+/- 12.8) for the naturally conceived group which were both within the normal range, and did not differ significantly (t = 0.265, df = 113, p = 0.791).
A one-way multivariate analysis of variance (MANOVA) was conducted using the two groups as the independent variable and the five Griffiths subscale scores

as the dependent variables. The omnibus test was significant (F(5,109)=6.456, p=.000).

The significant differences were for the Locomotor (PGD significantly lower) and Hearing and Language subscale (PGD significantly higher). When the analysis was conducted with maternal age included as a co-variate the difference on the Locomotor scale remained significant (F = 12.28, p = 0.001) but the difference on the Hearing and Language subscale became non-significant (F = 0.809, p=.307). This suggests that differences on the Hearing and Language subscale are largely accountable for by age maternal age differences between the cases and controls.

Table 4.2.9 Group differences on the overall and subscale scores on the Griffiths test.

	PGD	Natural Conception	p
Locomotor subscale (SD)	101.00 (14.19)	111.44 (14.39)	0.001
Personal social subscale (SD)	100.29 (18.92)	103.67 (16.58)	0.311
Hearing language subscale (SD)	106.42 (15.09)	99.86 (16.45)	0.030
Eye-hand subscale (SD)	100.66 (15.52)	102.61 (16.31)	0.519
Performance subscale (SD)	103.97 (16.51)	100.83 (19.70)	0.365
Griffiths General quotient (SD)	102.70 (13.13)	103.34 (12.81)	0.791

4.2.11 Child and Family Relationships

Parental questionnaires were used to assess family functioning.

Parents were posted the questionnaires once an appointment date had been fixed. They were sent out at least a week before the appointment. The parents were requested to complete the questionnaires before the assessment as far as possible. Any queries related to the questionnaires or their completion was addressed during the actual assessment. Parents were requested to bring the questionnaires with them for the appointment.

The set included the following questionnaires:

1. General Health Questionnaire: GHQ-28 (Goldberg and Hillier 1979)
2. The parenting Stress Index /Short form (Abidin 1990)
3. The Parental Acceptance and Rejection Questionnaire (Rohner and Khaleque 2005)

4. The Carey Temperament Scales (Second Edition): of which the following were used:
    a. Early Infancy Temperament Questionnaire for 1 to 4 month old infants.
    b. Revised Infant Temperament Questionnaire for 4 to 11 month old infants.
    c. Toddler Temperament Scale for 1-2 year old children.

This measure was used for children up to 35 months of age

5. Child Behaviour Checklist for ages 1½-5

This measure was used for 3 children who were over 35 months of age.

Response rate:

Overall there were 5 sets of missing questionnaires. One mother did not return any of the set. The other four had 1-3 of the questionnaires missing from the set. The response rate was 96%. A second copy of the set or missing questionnaires was sent by post. A further telephone reminder was attempted where possible. Thereafter it was taken as a non-response.

a. Child Behaviour Check List (CBCL)

Maternal reports of the child behavioural trends were obtained in 3 children from the PGD group. There were none in the same age range in the control group, so comparisons could not be made. The following is the report of the three children:

Table 4.2.10 Details of CBCL

Child	Internal	External	Other + Scale 5	Total
Case 29	3	0	3 + 1	7
Case 43	5	1	5 + 4	15
Case 110	3	4	0 + 1	8

This data could not be used in formal statistical analysis.

b. Carey Temperament Scales

There were no between group differences in the temperamental characteristics perceived by the mothers. A one-way multivariate analysis of variance (MANOVA) was conducted using the two groups as the independent variable and

the nine Toddler Temperament subscale scores as the dependent variables. The omnibus test was non-significant (F (9,98) = 0.565, p = 0.823) and all tests of between–groups effects were non-significant.

Table 4.2.11 Group differences on the overall and subscale scores on the Toddler Temperament test.

	PGD	Natural Conception	p
Activity	3.963 (.671)	4.035 (.645)	0.571
Rhythmicity	2.650 (.782)	2.575 (.684)	0.596
Approach	2.50 (.858)	2.76 (.970)	0.161
Adaptability	2.85 (.845)	3.03 (.842)	0.273
Intensity	3.722 (.772)	3.977 (.747)	0.088
Mood	2.698 (.640)	2.863 (.741)	0.228
Persistence	3.297 (.959)	3.418 (.681)	0.444
Distractibility	3.605 (1.162)	3.784 (.905)	0.373
Threshold	3.67 (.766)	3.62 (.836)	0.760

c. Parenting stress Index (PSI)

There was no difference in reported parental distress between the two groups.

A one-way multivariate analysis of variance (MANOVA) was conducted using the two groups as the independent variable and the three PSI subscales scores as dependent variables. The Omnibus test was non-significant (F (3,109) =1.075, p = 0.363). All tests of between–groups effects were non-significant. An independent groups t-test showed no significant difference between the groups in the total clinical score (t = 0.709, df =111, p = 0.480). The total clinical score was not entered into the MANOVA analysis as its value is derived from the sub-scale scores and therefore it would cause excessive co-linearity among the dependent variables.

Table 4.2.12 Group differences on the overall and subscale scores on the PSI test.

	PGD	Natural Conception	p
Parental distress	23.70 (7.19)	25.03 (7.83)	0.354
Parent-child dysfunctional interaction	15.96 (3.68)	15.89 (5.10)	0.934
Difficult child	20.70 (5.64)	22.27 (6.37)	0.174
Total clinical score	60.36 (13.41)	62.40 (16.43)	0.480

d. Parental Acceptance and Rejection Questionnaire (PARQ)

The PGD group had a significantly higher scores on the warmth-affection sub-scale, and significantly lower scores on the aggression-hostility and rejection sub-scales.

A one-way multivariate analysis of variance (MANOVA) was conducted using the two groups as the independent variable and the four PARQ subscales scores as dependent variables. The Omnibus test was significant ($F(4,107) = 4.107$, $p = 0.030$) and three tests of between–groups effects were significant.

Table 4.2.13 Group differences on the overall and subscale scores on the PARQ test.

	PGD	Natural Conception	p
PARQ warmth-affection	27.54 (7.94)	25.14 (4.28)	0.04
PARQ aggression-hostility	19.46 (5.21)	21.78 (5.77)	0.03
PARQ Neglect-indifference	17.73 (4.08)	18.83 (3.95)	0.15
PARQ Rejection	11.42 (2.84)	13.25 (3.65)	0.005

e. General Health Questionnaire (GHQ-28)

No differences were identified between the two groups using the GHQ-28, which was designed to detect current mental health problems in the general population. The GHQ-28 provides four dimensions, measuring somatic symptoms, anxiety and insomnia, social dysfunction and severe depression. Results were all within the normal range.

Table 4.2.14 Maternal GHQ Subscales

	PGD Mean (SD)	Naturally Conceived controls Mean (SD)	p value
Somatic Symptoms	1.59 (2.39)	2.00 (2.21)	0.52
Anxiety and insomnia	1.64 (2.40)	1.80 (2.24)	0.80
Social dysfunction	1.18 (1.89)	0.47 (0.81)	0.11
Severe depression	0.35 (0.94)	0.28 (0.71)	0.78
Total score	3.00 (4.06)	3.34 (4.51)	0.678

Chapter 5

5. Discussions

5.1 Discussion of the PORD study

5.1.1 Matching and socio-demographic details of study families

Maternal ages were similar in both the study and control groups of mothers. This reflects appropriate matching of the sample studied. This also reflects that women with well controlled mild to moderate renal disease, have uneventful obstetric histories which are comparable to mothers who do not have any renal disorder.

Families participating in this study with renal disease were more likely to be from socio-economically poorer backgrounds. Chronic kidney disease and the subsequent progression of the disease impose a relentless socio economic burden on the individuals and families (Norris & Agodoa 2005). Patients with chronic renal disease must cope with the demands of their occupation, the changes in their life roles, and the challenges and opportunities that life exposes them to while balancing the restrictions that chronic renal disease entails. It has been previously reported that socio-economic status has a significant impact on the incidence and treatment of end stage renal disease (ESRD) Socio-economic factors, such as low income, poor education, residence in a low-income areas, and consequently poor access to health care, are strong predictors for the development of ESRD (Norris & Agodoa 2005). Cukor *et al.* (Cukor *et al.*. 2007) have reported that socio-economic status has a significant impact on the incidence and treatment of chronic renal disease. Herd *et al.* (Herd, Goesling, & House 2007) reported that socioeconomic position is not simply a determinant of health differences but rather is a "fundamental cause" of these differences.

Key evidence is that the relationship between socio-economic position and health has not changed over time even as the intervening links between the two have. While the major causes of mortality have changed over the 20th century, from

infectious disease to chronic conditions, socio-economic health disparities have either persisted or increased(Pappas *et al.*. 1993).

What remains constant over time, across countries, and across individual lives is that socio-economic position shapes access to resources that help individuals avoid risk factors for disease and mortality(Phelan *et al.*. 2004) .

PORD mothers were as likely to have a university entry level education or a higher educational degree compared to control mothers.

Mothers of PORD children were more likely to be married than control mothers. This reflected that the study group were supported women who had mild to moderate renal disease which was well controlled. Several studies have demonstrated that social support is associated with improved outcomes and improved survival in several chronic illnesses, including cancer and end-stage renal disease (ESRD). The mechanism by which social support exerts its beneficial effects are unknown, but practical aid in achieving compliance, better access to health care, improved psychosocial as well as nutritional status and immune function, and decreased levels of stress may all play a role (Patel, Peterson, & Kimmel 2005). Nairman and colleagues (Naiman *et al.*. 2007)reported that being married in the pre-transplant period was associated with positive outcome for the graft, but not for recipient survival. When stratified by gender, the effect was present in males but was not seen in females, where neither graft nor recipient survival had an association with marital status.

The number mothers who smoked were fewer in the PORD group (none) compared to the control group. Current alcohol consumption was also less in mothers in the PORD group compared to mothers in the control group. Both smoking and alcohol consumption have been linked to reduced fertility. Thus this trend of reduced consumption of both alcohol and tobacco possibly reflects a lifestyle choice. Mothers with renal disease were already in a challenged situation, with ill health. It is more likely they will make healthier lifestyle choices; these may already have been promoted by their physicians.

Fathers of PORD children had a trend towards being more likely to smoke, while alcohol consumption appeared to be less common in this group when compared to the control group. The size of the groups did not provide any statistically valid information.

5.1.2 Health of PORD mothers in Pregnancy

PORD mothers reported more illnesses and obstetric complications during pregnancy.

Pregnancy complications are well documented in women with chronic renal disease depending on the severity of renal failure.(Chao *et al..*2002;Davison 2001;Davison & Bailey 2003;Holley & Reddy 2003;Hou 1999;Hou 2001;Hou 1994b;Jones & Hayslett 1996;Jungers *et al..* 1997;Lindheimer *et al..* 2007;Okundaye *et al..* 1998;Williams & Davison 2008). These range from raised blood pressure, proteinuria, urinary tract infection, polyhydramnios, deteriorating renal function, including significant rise of serum creatinine level, pre eclampsia and many suffer significant renal function loss during pregnancy, with an accelerated decline post-delivery. Mothers with renal transplants face added risks of graft rejection during pregnancy, graft dysfunction during pregnancy, which often persist post delivery sometimes leading to permanent graft loss. Bacterial and viral infections consequent to immunosuppression and toxic effects of immunosuppressant drugs are well reported in mothers who are transplant recipients (Report from EDTA1980;Armenti *et al..* 1998;Bar *etal.* 1996;Davison & Bailey 2003;Fuchs *et al..* 2007;McKay & Josephson 2006;Moon *et al..* 2000;Pahl *et al.* 1993;Sgro *etal.* 2002;Sibanda *et al..* 2007;Thompson *et al..* 2003;Toma *et al.*, 1999). Very little of the published data on illness and obstetric complications have been compared with a control group. The reported maternal complications in this study, though modest largely because of small number have shown similar complications reported in previous studies. Most important among these are hypertension pre- and post-pregnancy, recurrent urinary tract infections, and increased incidence of intervention in labour ranging from instrumental delivery to planned and emergency caesarean section. The increase incidence of illness and complications are accounted for by maternal renal disease and it was thus difficult to compare the significance of increased incidence of illnesses and obstetric complication between the two groups.

The use of medication was also highest in the PORD group. The differences were mainly accounted for by use of immunosuppressant medications that mothers with renal transplant were on and also antihypertensive medication that is more

commonly used in mothers with renal function impairment. These medications were an integral part of the treatment of renal disease. The use of medications like common analgesics, antacids, antibiotics and vitamins were similar in each group. Smoking in pregnancy was low in both PORD and control mothers even though it was lowest in the PORD group. None of the mothers, either in PORD group or control group were reported to consume alcohol in pregnancy. This reflects lifestyle choices made by both groups of women. Whereas women with renal impairment were possibly advised by health care professionals and better motivated taking into account their difficult health condition, women who were otherwise well, also appeared to be making this a lifestyle choice.

PORD mothers reported they were less likely to be supported by adults in pregnancy. This is apparently not in keeping with the fact that all renal mothers in the study were either married or cohabiting. However, the families with renal disease were from lower socioeconomic background and this could have affected the amount of social support available.

The PORD mothers were less likely to have spontaneous onset of labour when compared with the control group of mothers. The incidence of induction of labour was also higher in the PORD group. Incidence of caesarean sections, planned and emergency was higher in the PORD group. The increased incidence of planned caesarean section possibly reflects a cautious approach to the management of high risk pregnancy. The number of emergency caesarean section was also higher in the PORD group. This is likely to reflect maternal complications such as rising blood pressure often leading to pre-eclampsia, intrauterine growth restriction, deteriorating renal function, premature onset of labour and also fetal complications such as poor growth and fetal distress. There was also significantly higher number of instrumental deliveries in the PORD group. This reflects the increased level of interventions that mothers with renal disease received at delivery. Control mothers were most likely to have normal vaginal delivery. There are several studies which report increased caesarean section and instrumental deliveries in mothers with chronic renal disease.(Davison 2001;Holley & Reddy 2003;Hou 2001;Lindheimer *et al.*. 2007;Sibanda *et al.*.e 2007;Thompson *et al.*. 2003;Toma *et al.*. 1999;Williams & Davison 2008).

5.1.3 Neonatal results

PORD babies were more likely to be born after a caesarean section (planned and emergency) and control babies were more likely to be born by normal (non-instrumental) vaginal delivery. It has been discussed earlier that there is a well-documented increase in the rate of caesarean section in pregnant mothers with renal disease.

The PORD group of infants had a significant lower gestational age and higher proportion with gestation age less than 37 weeks, ($p<0.01$). The PORD group also had lower birth weight, and higher proportion with birth weight less than 2500g. Previous studies have reported an increase in prematurity and lower birth weight in children born to mothers with renal disease. (Davison 2001;Holley & Reddy 2003;Hou 2001;Lindheimer *et al.*. 2007;Sibanda *et al.*. 2007;Thompson *et al.*, 2003;Toma *et al.*. 1999;Williams & Davison 2008). A lot of the infants in this study were late preterm infants (34-36 weeks gestation). Research in neonatology so far has looked into epidemiological, clinical and follow-up aspects of very low birth weight newborns who usually have a gestational age less than 34 weeks and high morbidity and mortality rates. Only recently has attention turned to the issues of preterm infants with higher gestational ages and the expression 'late preterm' has been adopted to classify newborns with gestational ages between $34^{0/7}$ and $36^{6/7}$ weeks (Escobar *et al.*. 2006).

PORD children were more likely to require resuscitation after birth when compared to the control group, though the difference was not statistically significant. There is a correlation between caesarean section, prematurity and increase in requirement for resuscitation. Parents of PORD children and the medical staff responsible for managing the pregnancy may be more anxious about the outcome and intervene in labour at an earlier stage or plan delivery by caesarean section. Many previous studies (Alsaran & Sabry 2008;Davison & Bailey 2003;Holley & Reddy 2003;Hou 2001;Moon *et al.*. 2000;Okundaye *et al.*., 1998;Reddy & Holley 2007;Sgro *et al.*., 2002;Sibanda *et al.*. 2007;Thompson *et al.*. 2003;Toma *et al.*. 1999;Williams & Davison 2008) have reported the increased incidence of premature birth and subsequent admission to a neonatal unit. Pregnancies in mothers with renal disease are often complicated by maternal

high blood pressure, pre-eclampsia and eclampsia, deteriorating maternal renal function, compromised graft function of transplanted kidneys, IUGR, poor placental function and pre-term labour. It is obvious that in such situations there may be a need to intervene and thus the increased incidence of preterm labour, prematurity and increase in resuscitation. A recent study from Brazil by de Almeida and colleagues (de Almeida *et al.*. 2007) reported that the chance of needing any resuscitative procedure were twice as high in the late preterm group compared with the full term group. They also go on to report, that with other variables controlled for, being a late preterm infant doubles the chance of receiving bag and mask ventilation in the delivery room (de Almeida *et al.*, 2007). This group has been identified as a high risk group, who are more likely to receive resuscitative facilities in the immediate neonatal life. A significant proportion (8%) of this group required supplemental oxygen support for at least 1 hour, almost 3 times the rate found in infants born at ≥ 37 weeks (Escobar *et al.*, 2006).

In this study the proportion of babies admitted to the neonatal unit was different in the two groups. 8 babies (33%) born after PORD were admitted to the neonatal unit compared to 3 babies in the control group (8%). This difference was significant, $p<0.01$.

The number of days spent in the neonatal unit appeared to differ in the two groups but there were inadequate numbers to compare the significance of stay in neonatal unit in terms of length of stay ≥ 7 days.

Ventilation appeared more common in the PORD group. Four babies (17%) born in the PORD group were ventilated, compared to one (3%) in the control group. The difference was approaching significance ($p=0.052$). Two of the babies in the PORD group were ventilated for 1 and 2 days respectively because of prematurity. The ventilatory support required was minimal. The other two babies were ventilated for 15 and 30 days. These two babies were premature and had respiratory distress and sepsis. The baby in the control group who was ventilated was delivered prematurely at 31 weeks by emergency section secondary to maternal placental abruption and was only ventilated for half a day. These findings agree several previous studies where premature babies were more commonly ventilated compared with controls.

Reasons for admission to a neonatal unit included a range of common neonatal problems. There was difference between the two groups. In the control group 10 (27%) of babies had documented neonatal illness compared to 3 (12.5%) babies in the PORD group. The types of illnesses that affected the children as neonates also varied. In view of the fact that there was no increase in neonatal illness in the PORD group (actually there were fewer reported neonatal illnesses), the increased admission to the neonatal unit is most likely due to the increase in prematurity and its effects. It may also simply reflect increased parental and/or physician anxiety. The number of children in the study was too few to provide statistically robust outcome data.

Mothers from the PORD group were less likely to exclusively breastfeed their babies than mothers from the control group. Nine (39%) mothers from the PORD group did not exclusively breast feed compared to 4 (11%) mothers in the control group, $p < 0.01$.

Of the mothers who chose to breast feed, the length of exclusive breast-feeding was similar between groups. One of the most common reasons for this is that a lot of the mothers with renal disease (especially the transplant recipients) were often strongly advised against breast-feeding, because of the medications they were on. The European Best Practice Guidelines (2002) (European Best Practice Guidelines 2002a)recommends that immunosuppressive therapy based on cyclosporine or tacrolimus with or without steroids and azathioprine may be continued in renal transplant women during pregnancy. Because of drug transfer into maternal milk, breast feeding is not recommended. A case report by Munoz-Flores-Thiagarajan (Munoz-Flores-Thiagarajan *et al.*. 2001), reports that an infant of a cyclosporine treated mother was breast-fed exclusively during the first 10.5 months of life and did not absorb a detectable amount of the drug. Fetal growth and development were normal. Moretii *et al.* (Moretti *et al.*. 2003)(2003) reported that during recent years, cases of uneventful pregnancies and subsequent successful breast-feeding have been reported in women on cyclosporine therapy. The infant's blood cyclosporine concentration has usually been very low. Based on these findings and the lack of detectable adverse effects, some investigators have suggested that women on cyclosporine may breast-feed; challenging the conventional view that cyclosporine is contraindicated during breast-feeding.

Similarly possible safety of tacrolimus while breast feeding in transplant recipient mothers have been reported in several studies (French *et al.*. 2003;Gardiner & Begg 2006). Based on these findings and the lack of clinically evident adverse effects, the conventional view that most immunosuppressants used in transplant recipient mothers is contraindicated during breast-feeding, has been challenged (Nyberg *et al.*. 1998;Thiru, Bateman, & Coulthard 1997). However, Moretti *et al.* (Moretti *et al.*. 2006)also states that whether breast-feeding exposure, and intrauterine exposure, to immunosuppressants like cyclosporine and tacrolimus alters the immunologic function of the infants awaits further study. The CARI guidelines (Grimer 2007) also supports the findings that levels of immunosuppressant medication in breast milk and in infant serum is low and thus many investigators are continuing the medications but there are no clear guidelines as to whether the risk to the infant in breast-feeding is low enough to justify the potential benefits of breast feeding. The most recent report from the National Transplantation Pregnancy Registry (NTPR) by Armenti *et al.* (Armenti *et al.*. 2005) stated that breast feeding for female transplant recipient mothers remains an area where recommendations are evolving. The report stated that mothers were on Sandimmune, Neoral, Tacrolimus for durations ranging from a few days or weeks to one to two years. At last follow up there were no reports of problems related to breast feeding in the children. In 1987 B L Asselin (Asselin & Lawrence 1987)suggested that mothers who have normal or mildly decreased renal function can breast feed successfully, whereas mothers with moderate renal insufficiency can breast feed if infant and mother were well enough in stable condition, thus raising a possibility that there was a risk that health of mother and infant might be at risk. Asselin (Asselin & Lawrence 1987)stated that mothers with severe renal insufficiency should not breast feed. However, the author suggested that renal transplant mothers most like produced immuno-competent milk and very little azathioprine was present in breast milk. It was reported that the mothers on antihypertensive medication can also successfully breast feed if their management is fine tuned (Asselin & Lawrence 1987). Still decades later guidelines are slowly changing or haven't changed. The American Association of Paediatrics supports breast feeding in mothers on prednisolone, advises against breast feeding by those on cyclosporine and provides no recommendations regarding tacrolimus and azathioprine (McKay & Josephson 2006).

Previous studies have shown that mothers who were excluded from breast feeding often found it difficult to adjust to and most would actually have breast fed. This may have affected that women's perception of her mothering skills further increasing the anxieties which are commonly associated with chronic illness (Cukor *et al.*. 2007;Willis *et al.*. 2000).Thus in the current climate of promotion of breast feeding and also due to social pressures more and more mothers on immunosuppressants will choose to breast feed.

Another reason for reduction in exclusive breast feeding in the PORD group could be due to difficulties in establishing breast feeding after caesarean section (Dewey *et al.*. 2003a). Some of the mothers were unwell after pregnancy and found it difficult to establish breast feeding. Practical difficulties such as mother being admitted to the hospital ward, high dependency unit and intensive care unit may affect breast feeding. Often these areas are not geared to promote ideal breast feeding practices. Many of the babies born in the PORD group were premature and admitted to a neonatal intensive care unit. Maternal ill health together with babies being admitted to the neonatal unit often prevents bonding and may lead to difficulties establishing breast feeding. Breastfeeding a premature infant can be an additional challenge for a mother. Initiation of breast feeding is often delayed in preterm babies. Successful breastfeeding of these infants also depends on many other factors, including the mother's physical capacity to produce milk (mothers ill health often being an important cause of inadequate milk), and the family, institutional and nursing assistance received.

5.1.4 Physical assessments

Hospital admissions after the neonatal period were similar in the PORD group compared to the control group. This reflects that children born to mothers with renal disease were in no way more prone to illness when compared with controls. This finding is supported by data from a previous study by Korsch *et al.* (Korsch *et al.*. 1980)where health of children born to mothers with renal disease during the first year of life was reported as excellent with no documented unusual intercurrent illnesses, metabolic disorders or other physical problems. In another follow up study of children born to renal transplant mothers, Willis and colleagues (Willis *et al.*., 2000)reported no abnormal medical illnesses. Only minor

childhood problems like otitis, urinary tract infection, tonsillitis and a minor squint not requiring surgery were reported. None of these studies compared the renal children with a control group. There is no published follow up data relating to children who are born to mothers with different levels of renal impairment but not transplanted. The present study included 16 women whose renal function varied from near normal to mild to moderate impairment. Previous studies do not report any follow up data regarding children born to women with renal disease.

Frequencies of illnesses reported by parents were higher in the control group. Korsch (Korsch *et al..* 1980) reported that parents with renal disease were more inclined to seek immediate medical attention for minor illnesses in their children, based on the "philosophy of taking no chances". Willis and colleagues (Willis *et al..* 2000) in their follow up study reported increased maternal anxiety about their children's health. Our study findings are distinctly different from these two previous studies. However both the previous studies involved renal transplant mothers and their children, while our study mothers had different levels of renal function impairment. It is possible that renal transplantation is associated with increased maternal worry compared to our mixed group of patients. Illnesses were classified into respiratory tract infections, common childhood infections, fractured bones, gastrointestinal, atopic (eczema and asthma) and other illnesses. Of these, there was trend towards control group children having more atopic illness, chicken pox, common childhood infections and fractured bones than the PORD group. This trend was not significant. It is possible that there were subtle differences relate to differing parental attitudes to reporting illnesses. Childhood medications were similar between the groups. This further supports previous suggestions that there is no increase in the use of medications in 'PORD' children. This could be objectively viewed as evidence that children from both groups had similar rates of illness.

There was no difference between the two groups in need for surgery.

Findings of general examination of both the groups did not reveal any major abnormalities. Three control group children had undescended testes, asymptomatic tight foreskin and eczema respectively. Two children from the PORD group had mild eczema.

Findings from previous studies of follow up of children of renal transplant are summarised in table 2.17. A study by Willis *et al.* in 2000 looked into the physical status and developmental outcome of children born to mothers following renal transpalntation (Willis *et al.*., 2000). This was a cross-sectional, partially controlled cohort survey of 48 children born to 34 women transplanted at a single centre from 1971 to 1992. Data on maternal renal disease, immunosuppression, pregnancy, delivery and child development were collected using hospital records and parental questionnaire. Children underwent physical examination, urinalysis and urinary tract ultrasound examination (US). The developmental assessments considered the child's developmental milestones, scholastic and educational achievement. A single urine sample was collected for routine urinalysis, and if abnormal, for microscopy and bacteriological examination. Ultrasound examination of the child's urinary tract was performed by the one ultrasonographer (one of the investigators). Micturating cystourethrograms (MCUG) were not performed due to their invasive nature and to the wide age range of the children studied. This was the only study which has looked into the long term outcome of the children born to mothers with renal transplant. The authors concluded that the neonatal outcomes were generally good in the study group despite the high incidence of risk factors such as prematurity and growth retardation. The authors were unable to identify any pattern of fetal abnormalities related to the use of maternal corticosteroids, azathioprine or cyclosporin A. However the possibility of further problems remains. The children in the study though born premature and small demonstarted good catch up growth. The authors thus concluded that postnatal progress was not adversely affected by maternal renal function or maternal immune suppressive medication. Child development and general health were normal in the study group but there was no direct control group to compare. However this study raises numerous psychological issues arising in families where the mother has undergone renal transplantation, which may influence the development of a child. These issues may be of great significance but have so far not been highlighted in any published studies. Barbara Korsch *et al.* from the Dialysis and Transplant Programme at the Children's Hospital of Los Angeles published a study in 1980 of physical and psychological follow-up of offspring of renal allograft recipients (Korsch *et al.*., 1980) All ten children were in good health at the time of follow up. Growth in all

the children was normal. Results of the developmental tests show that all children tested were within the normal range

There are some important observations available from the interviews with families relating to parenting from this study:

a. The majority of children were developing normally without any obvious behavioral problems.
b. Their upbringing was not affected by the graft recipient status of their parent.
c. Parents noted that there was a trend to seek immediate medical attention for minor illnesses in their children "based on a philosophy of taking no chances

The findings in this study are very similar though the mothers had different degrees of renal impairment and not only renal transplant.

5.1.5 Growth

There were no differences between the groups for growth in terms of actual height in cms and weight in kgs and centiles. In the PORD group the mean weight in kilograms (SD) was 16.98 (7.67) while that for the control group was 15.34 (4.23), not reaching statistical significance. In the PORD group the mean height in centimetres (SD) was 99.55 (21.34) while that for the control group was 97.72 (13.79), which were not statistically significant. Sgro *et al.* (2002) (Sgro *et al..* 2002)reported that the children born to renal transplant recipient mothers were significantly heavier for age, and were also significantly shorter for age compared to the control group. Willis *et al.* reported that current height and weight was on the 50th centile. Korsch *et al.* (Korsch *et al..* 1980)reported that three children were below 25th centile for weight but all were above 25th centile for height. It is difficult to compare the current study data with those mentioned mainly because they studied a select group of children born to renal transplant recipient mothers and also the age range at assessment varied.

5.1.6 Congenital malformation

There was no reported congenital malformation, major or minor either in the PORD or the control group. This was most likely because of the small sample size. Follow up studies by Sgro *et al.* (2002)(Sgro *et al..* 2002) and Willis *et al.*

(2000) (Willis *et al.*. 2000) reported some minor and major congenital anomalies. Sgro *et al.* (Sgro *et al..* 2002)reported bilateral clubfeet, imperforate anus and hypospadias (all in a single child) and Willis *et al.* reported epigastric hernia, hydrocoele, cystic hygroma, claw hand, obstructive uropathy, pulmonary hypoplasia and hydronephrosis. Korsh *et al.* (Korsch *et al..* 1980)did not report any anomalies in their series.

5.1.7 Griffiths Mental Developmental Scales

The data indicated that there was little difference between the groups .The mean Griffiths quotient was 106.29 (SD 18.48) for the PORD group and 105.65 (SD 11.60) for the control group which were both within the normal range, and did not differ significantly. Sgro *et al.* (2002) (Sgro *et al..* 2002)in their series assessed development using the Denver Development Screening Test. Some families who were unable to attend were interviewed over the phone, with the actual children not being directly assessed. They reported one child with moderate to severe hearing loss (requiring a hearing aid), one child with learning disability and one with pervasive developmental disorder. Willis *et al.*(Willis *et al..* 2000) mentioned that 98% of their series had normal development (details of assessment not stated in the report). The single child with developmental delay in their series was one of the triplets with cerebral palsy. The children's development in the study by Korsh *et al.* (1980) (Korsch *et al..* 1980) was carried by a specialist in assessing child development. The Stanford-Binet test was used for the two oldest children and Gesell Developmental schedules and Bayley Scales of Infant Development was used for children under 30months of age. All children tested were within the normal range.

5.1.8 Child and Family Relationships

1. Parental questionnaires to assess family functioning

Overall there were 4 sets of missing questionnaires. This was an 83 % response rate amongst those who attended. This reflects a good response rate for returning of questionnaires. The questionnaires were sent to the parents at least a week prior

to the appointment and collected from the parents at the appointment. This seemed a practically reliable method of collecting the questionnaires. Recent studies by Edwards *et al.* report that contacting participants before sending questionnaires and follow up contact significantly increased response rates (Edwards *et al.*. 2002;Edwards *et al.*. 2007). The high response rate from both the groups may be a courtesy effect: the parents of PORD children were willing to cooperate because of the medical support provided by hospitals and the parents of the control group were willing because their child received a thorough paediatric assessment.

2. Analysis

The power analysis for the PORD study indicated that the likelihood of detecting any differences or association would be low. Individual t-tests and chi-squares were employed, as the power of larger multivariate test would be too low. Therefore caution should be exercised in the interpretation of the PORD results as the low power may lead to a Type II error (failing to find a significant effect that exists in the population) and the multiple tests may lead to a Type I error (finding a significant effect due to chance).

3. Child socio emotional behaviour

a. The Child behaviour check list (CBCL) 1 ½ - 5 and 6-18

The Child behaviour check list (CBCL) 1 ½ - 5 and 6-18 which provides a standardised rating and descriptive details of children's functioning as seen by parents showed significant differences between the PORD and the control group. The mothers in the PORD group reported a significantly higher perception of externalising problems like rule breaking and aggressive behaviour in their children. The PORD mothers also reported a significantly higher perception of other problems when compared with the control group. The total scores of the CBCL were also higher in the PORD group reflecting that these mothers perceived difficulties in their children's functioning more commonly. The sample size of the study was not large enough to provide any statistically significant data,

however the finding is interesting as one of the hypotheses of the study was that there would be greater occurrence of difficult temperament and emotional or behavioural problems in children born to mothers with chronic renal disease

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b. Carey Temperament Scales

The Carey Temperament scales did not report any difference in child temperamental characteristics perceived by mothers in either group. Thus the mothers with chronic renal illness, in spite of stresses of their illness did not perceive their children any differently from mothers who were well. Previous studies have also suggested that child temperament development is not significantly affected by parental ill health.

Chronic illness is highly prevalent among adults many of whom have children and parental illness may or may not have an impact on affected families. The existing literature has provided a framework for how parental illness may influence child functioning and normal development (Armsden & Lewis 1993;Rolland 1999). Controlled studies examining functional outcomes for children whose parents had either a chronic or acute medical illness have yielded mixed results. Hirsch *et al.* (Hirsch, Moos, & Reischl 1985)found that children with an arthritic parent had poorer self-esteem than controls but displayed no differences on measures of psychiatric symptoms or adjustment. Harris and Zakowski (Harris & Zakowski 2003) found no differences between children with or without a parent who had cancer. As expected, more pronounced differences have been demonstrated for children with terminally ill parents (Siegel 1992). Armistead *et al.* (Armistead, Klein, & Forehand 1995) concluded that parental illness does influence child functioning and may be particularly associated with internalizing symptoms. However, differences between children with and without a sick parent are small such that children with sick parents appear within normal limits on measures of outcome (Steele, Forehand, & Armistead 1997) .

Findings thus far have suggested that differences between children with and without a sick parent are minimal, but there are individual and familial moderators of outcome. Potential moderators of differences included maternal distress, parenting variables (aggravation and warmth), functional impairment related to

illness, and demographic characteristics. (Annunziato, Rakotomihamina, & Rubacka 2007).

4. Parental well being and family functioning

No significant differences were found on the three Parental Stress Index subscales and total scores. There was also no difference in the total GHQ scores. Thus so far contrary to our expectations mothers with chronic renal disease seem to cope with the extra stresses of chronic illness without evidence of ill effects on their well being and relationships.

It was a reassuring finding that chronically ill mothers were facing up to challenges of parenthood as ably as well mothers.

Parent –child relationship

There was no difference in the overall and subscale scores of Parental acceptance rejection scores between the PORD and control groups.

The extent, kind and timing of incapacitation faced by the mother will affect the degree of family stress. Outcome of the illness that the woman is suffering from is also closely related to the family functioning. The course of any chronic illness can be progressive, constant and relapsing/episodic. Families are stressed with both the frequency of transition between crisis and non-crisis and children particularly are affected by the uncertainty of the course and outcome of maternal health. The women with chronic illness are themselves sufferers of the above stresses and anxieties.

Additionally children have a great ability to sense danger (to their mother's health) and threat of loss despite very few actually communicating such fears and worries (Rolland 1999).

5.1.9 Strengths of the study

1. Comparison groups

Two groups were chosen, the children born after maternal chronic renal disease (PORD) and a control group of children born to mothers who did not have any reported renal impairment. The control group provided a standard of 'normal' by which to compare the PORD children. These children were from the same geographical area and in this way attention can also be given to the family structure and educational status of the parents.

2. Outcome measures

The developmental assessment used in this study was the Griffiths Mental Development Scales from birth to 2 years (1996 Revision) and the Griffiths Mental Development Scales from birth to eight years (1984). The Griffiths Mental Development Scales from birth to 2 years re-standardised in the United Kingdom in 1996 to reflect the child rearing practices, social habits, racial groupings and other socio-demographic factors pertinent to modern day Britain. The extended 2-8 years Griffiths scales (subsequently published) were undergoing the most recent update and re-standardising for a fairly long time and was not used in this study due to delays in publication of the latest testing scales and validation of data. The project would be delayed if the latest scales were to be used.

3. Single paediatrician

All the children were assessed by the same paediatrician (IB), providing consistency in assessments and negating the inter observer bias.

4. Personal contact and advice lines

After the assessments the parents had a chance to discuss any queries or definite concerns with a paediatrician. The parents appreciated this and many were reassured. It is worth noting that families who participated in this preliminary

study expressed gratitude that their child had been formally assessed indicating an unfulfilled medical need, which is likely to provide an acceptable participation rate for larger studies- always a challenge in research of this nature.

5. The nature of the study

This preliminary study was the first detailed control matched study published on children born to mothers with chronic renal disease. Although the number of cases is small the study does provide provisional reassurance that these children do not have any overt problems over and above those known to be a risk from being born early and with a low birth weight. This preliminary study showed that the methodology is viable to assess a larger group of children.

5.1.10 Weaknesses of the study

1. Participation rate

Of the initial 80 children found to be eligible only 35 (44%) responded to the invite and finally 24 (30%) of the eligible children were assessed. We identified this weakness at the study progressed. The following reasons were possibly responsible:

- 11 families (14%) even though they responded initially, later did not participate. Restriction s on the ethical approval meant we were not allowed to investigate reasons of non participation but some mothers wrote that even though they saw the need for the research and morally agreed they personally found it not suitable to participate. There is a possibility that these mothers had a worry about the consequences of their illness on the child and did not want to know about it any further. It is also possible that they perceived their children as well and did not want to delve any deeper into the issues in case a problem did emerge. Every effort was made to contact these families, to arrange discussions which might have cleared doubts and fears but it was often practically difficult to do so.

- Families were less reluctant to reply to an invitation from a Paediatric department with whom they had no contact. It was thus felt that an invitation from the Renal team would be more appropriate. But endeavours at working on this failed due to a severe shortage of man power in the renal team and it proved not possible to go forward with this plan.
- Some families were under the care of the Renal team as their tertiary care centre and were still mainly under their local hospital on a shared care basis. They were attending the Royal Free Hospital only for annual or biannual follow up. Such families found it difficult to attend appointments even if flexibility of venue, date and time were provided.

2. Observer bias

The paediatrician was not blinded to the maternal disease status. This was for practical administrative reasons. At the conception of the study it was thought that recruitment could be organised by a renal research nurse. For practical difficulties this was not possible to organise. The paediatrician was involved in recruitment of children in to the study and arranged appointments. Although the paediatrician examined and assessed the children in a standardised manner, it would have been more ideal if the paediatrician was also blinded to the maternal disease status. However the lack of blinding did give the paediatrician the chance to discuss and advice parents who had specific concerns about their child's health.

3. Missing cases

An attempt was made to collect information from all families that had been recruited, but did not attend. However, in a proportion of cases, families were either uncontactable due to change of address or contact details or the family declined to participate. Due to ethical constraints we were not able to ascertain causes of non participation. Families who had shown interest initially were often unable to attend due to the distance they required to travel; the family sometimes didn't attend mutually arranged appointments, mother giving birth to new baby in family, ill health of mother and/or child etc.

4. Sample size

The sample size was small and was not adequate to obtain statistically reliable outcomes for most of the outcomes measured. Although the study was not adequately powered for definite conclusions, it nevertheless provided reassuring data that there was no evidence in the children born to mothers with chronic renal disease of major health concerns, effects on the mother child relationship or neurodevelopment scoring. It also provides a possible model for future studies.

5. Growth data

The children were assessed at different ages and thus there was the variability of difference of age at assessment. This could have been overcome by a common statistical way of standardising data on one scale so a comparison could take place. One way to achieve this was to use z-score so that the variability of assessment at different age could be overcome.

5.2 Discussion for PGD study

5.2.1 Matching and socio demographic details of study families

Maternal and paternal ages were higher in the PGD group than the control parents. This is not surprising as many parents who seek assistance to conceive will have had a period of unsuccessful attempts to conceive naturally before treatment will be considered. It is also well recognised that older mothers have reduced fertility and may be more likely to require assistance to conceive. There is a definite increase in the number of women bearing children in the 30- and 40-year-old age groups. In the United States this is projected to increase 42% and the percent of births to this age group is projected to increase 37%. This is apparently because of a trend to postpone childbearing and first birth due to women's career priorities, advanced education, control over fertility, financial concerns, late and second marriages, and infertility. Associated with this is an increase in visits to fertility specialists by older women who have an intrinsic decrease in fecundity with advancing age(Gindoff & Jewelewicz 1986;Lansac 1995).

The majority of PGD parents were from social class higher than Class 3. This finding has been noted before (Bowen *et al.*. 1998;Sutcliffe *et al.*. 2001). The social class of parents of control group in this study was well matched. There was no difference between groups for social class of mothers or fathers. Control group mothers were as likely to have a university entry level education and higher educational qualifications as PGD mothers.

Mothers of PGD children were more likely to be married than control mothers. Couples are not usually offered investigations for fertility unless they have had at least one year of failure to conceive naturally. Parents who are subsequently investigated and conceive after assisted reproductive techniques are thus more likely to be in a stable relationship compared with parents who conceived naturally.

The number of mothers who smoked was fewer in the PGD group (none) compared to the control group. Current alcohol consumption was also less in the mothers in the PGD group compared to the mothers in the control group. Both smoking and alcohol consumption has been linked to reduced fertility. Thus this

trend of reduced consumption of both alcohol and tobacco possibly reflects a lifestyle choice.

There was no significant difference between the numbers of children that the fathers in each group had from previous relationships. However, there was a trend towards PGD fathers having two or more children from previous relationships.

Fathers of PGD children were more likely to consume alcohol, while smoking was less common in this group when compared to the control group. Smoking in males has been demonstrated to reduce fertility (Hassan & Killick 2004). While the reduced smoking possibly reflects a lifestyle choice made by the PGD fathers, an increased alcohol consumption has been reported in previous studies (Peters 2005).

5.2.2 Health of PGD mothers in Pregnancy

PGD mothers reported more illnesses and obstetric complications during pregnancy. This increase can be accounted for by ovarian hyperstimulation syndrome. This syndrome is recognised to be a complication of the assisted reproductive technology (ART) procedures to stimulate egg development (Olivennes 2003). Thus it is not possible to compare the incidence of these symptoms in the PGD mother when compared with the naturally conceived group.

Even though placenta praevia and associated increased incidence of bleeding is a recognised complication of embryo implantation processes (Love & Wallace 1996;Romundstad *et al.*. 2006;Tan *et al.*. 1992;Verlaenen *et al.*. 1995) it was not reported in this study. This perhaps reflected the relatively small number of cases. Previous studies have also reported an increase in vaginal bleeding and hypertension (2008;Romundstad *et al.*. 2006;Tan *et al.*. 1992), but no increase in hypertension was found in this study.

The use of medication was also highest in the PGD group. The differences were mainly accounted for by use of progesterone, aspirin, steroids (hydrocortisone and prednisolone), heparin and immunoglobulins. These medications were an integrated part of the IVF cycles. The use of medications like common analgesics, antacids, antibiotics and vitamins were similar in each group.

Smoking in pregnancy was low in both PGD and control mothers even though it was lowest in the PGD group. None of the PGD mothers were reported to

consume alcohol in pregnancy. The rate in the control group was 16.6%. This possibly reflected the desire of the PGD group of mothers to create an optimal environment for a pregnancy, which wouldn't be surprising taking into account the history of infertility.

PGD mothers were more likely to be supported by adults in pregnancy. This could be related to the fact that more mothers in the PGD group were married. It might be that members of stable social support network are more eager to help couples with already a difficult history of infertility.

The PGD mothers were less likely to have spontaneous onset of labour when compared with the control group of mothers. The incidence of labour being induced was not different. Incidence of caesarean sections, planned and emergency was higher in the PGD group. The incidence of planned caesarean section was particularly high possibly reflecting a cautious approach to the management of high risk pregnancy. There was no significant difference in occurrence of instrumental deliveries. Control mothers were most likely to have a normal vaginal delivery. An increased rate of caesarean section after IVF has been reported in several studies (2008;Allen *et al.*. 2006;Buckett *et al.*. 2007;Dhont *et al.*.1999;Romundstad *et al.*. 2006;Sermon *et al.*. 2007), but this increase in emergency section and dysfunctional labour may be secondary to the increased maternal age in the ART groups rather than the assisted reproductive process per se (Berkowitz *et al.*. 1990;Cohen, Newman, & Friedman 1980). However, some studies where maternal age was controlled for, still report increased incidence of caesarean section (Maman *et al.*. 1998;Ochsenkuhn *et al.*. 2003;Reubinoff *et al.*. 1997), so this finding might reflect obstetric (over)-caution.

5.2.3 Neonatal results

PGD babies were more likely to be born after a caesarean section: planned and emergency and control babies were more likely to be born by normal (non-instrumental) vaginal delivery. As discussed earlier there is a well documented increase in the rate of caesarean section after IVF.

The PGD group had a significant lower gestational age and higher number with gestation age less than 37 weeks, (p=0.01). The PGD group also had lower birth weight, and higher proportion with birth weight less than 2500g. Previous studies

have reported an increase in prematurity and lower birth weight in children born after ART (2008;Dhont *et al.*. 1999;Gissler & Hemminki 1996;Koivurova *et al.*. 2002a;Leslie *et al.*. 1998;Petersen *et al.*. 1995;Wennerholm & Bergh 2000).

PGD children were not more likely to require resuscitation after birth when compared to the control group. It is possible there is a correlation between the increase in caesarean section and the decreased need for resuscitation in this group. Parents of PGD children and the medical staff responsible for managing the pregnancy may be more anxious about the outcome and intervene in labour at an earlier stage or plan delivery by caesarean section. There is a possibility that this cautious approach negated the need for later resuscitation. The number of babies admitted to the neonatal unit was different in the two groups. 8 babies (16%) born after PGD were admitted to the neonatal unit compared to 7 babies in the control group (10.6%). This difference was not statistically significant $p = 0.391$. The number of days spent in the neonatal unit did not differ between the two groups. There were inadequate numbers in the two groups to compare the significance of length of stay of ≥ 7 days in neonatal units. Mechanical ventilation occurred more commonly in the control group; however the difference was not significant. Two babies (4%) born after PGD were ventilated; one was ventilated for 4 days because of septicaemia and meningitis and the second because of meconium aspiration — the duration could not be recalled by the parents. Three babies in the control group was ventilated, one for transient tachypnoea of the new born (TTN) (duration not recalled), one for meconium aspiration for 5 hours and the last could not recall the duration or the reason for ventilation. Reasons for admission to the neonatal unit included a range of common neonatal problems. There was no difference seen between the groups. The types of illnesses that affected the children as neonates were also varied. These illnesses did not necessarily require admission to the neonatal unit. In view of the fact that there was no increase in neonatal illness in the PGD group the increased admission to the neonatal unit may simply reflect an increase in parental or physician anxiety. Even though the study was not powered by significant numbers the findings support a previous study that also found no difference in neonatal outcomes between IVF and naturally conceived children (Leslie *et al.*. 1998).

Mothers from the PGD group were more likely to begin exclusive breastfeeding of their babies than mothers from the control group. Of the mothers who chose to breast feed, the length of exclusive breast feeding was similar between groups. This possibly reflects greater motivation from the PGD mothers. Previous findings often suggested decreased rate of breast feeding in IVF mothers compared to mothers of naturally conceived children (Leslie *et al.*. 1998;Peters 2005). The reason for reduction in exclusive breast feeding in IVF groups could be due to difficulties in establishing breast feeding after caesarean section (Dewey *et al.*. 2003b) or to more advanced maternal age. However in this study in spite of increased caesarean section, the PGD mothers were more likely to begin exclusive breast feeding. This could reflect current increased awareness among both parents and medical professionals in promoting the practice of breast feeding. It could also reflect the awareness of the belief that breast feeding promotes better maternal child bonding about which the PGD group of mothers could have been concerned.

5.2.4Physical assessments

Hospital admissions were less common in the PGD group compared to the control group. The number of illnesses reported by parents was similar in each group. Illnesses were classified into upper respiratory tract, lower respiratory tract, dermatological, gastrointestinal, atopic and other illnesses. Of these, there was trend towards less illness in the control group for gastrointestinal and atopic complaints. This trend was not significant. It is possible that differences were secondary to subtle differences in parental attitudes to reporting illnesses. Use of childhood medications was similar between the groups. This further supports the view that there was no increase in use of medications in the 'PGD children' and the control group. This could be objectively viewed as evidence that children from both groups had similar rates of illness as the no of reported illness and use of medications are not different in the two groups.

There was a slight difference between the two groups regarding type of surgery although rates wee similar. The children in the PGD group required minor surgical procedures like circumcision and pyloromyotomy and the control group

also required similar procedures like minor plastic surgical procedures and corrective surgery for club foot.
Findings of the general examination of both the groups did not reveal any abnormalities apart from a single control child with hypospadias.

5.2.5 Growth

There were no differences noted between the groups for childhood growth in terms of actual height in cms and weight in kgs and centiles. There was no previous data relating to the growth of children born after PGD beyond birth. However, the hypothesis that the overall health of children born after PGD should be similar to that of children born after ART is supported by data from this study. These findings support a previous study of children born after assisted reproduction and found that the childhood weight was no different between groups (Saunders *et al.*. 1996). This is reassuring in view of the recent concerns relating to an increase in Beckwith - Wiedemann syndrome in IVF conceived children (DeBaun, Niemitz, & Feinberg 2003;Maher *et al.*. 2003;Sutcliffe *et al.*. 2006) and evidence of overgrowth in other mammals after assisted conception (Khosla *et al.*. 2001;McEvoy *et al.*. 1998). This study provided some initial data about the physical well being of children born after PGD. However numbers were too few to provide statistical significance. Further bigger studies would be useful to provide such data.

5.2.6 Congenital malformation

There was no difference between the groups for incidence of congenital malformations, major or minor. Two children (4%) from the PGD group had reported minor malformations (strawberry naevus and abnormality to the shape of the pinna) and 3 children (5%) from the control group reported two major abnormalities (Duane's syndrome, a congenital malfunction of eye muscles, and hypospadias) and one minor (plagiocephaly).

5.2.7 Griffiths Mental Developmental Scales

The data indicated that there was little difference between the groups .The mean Griffiths quotient was 102.7 (+/- 13.1) for the PGD group and 103.3 (+/- 12.8) for the naturally conceived group which were both in the normal range, and did not differ significantly ($t = 0.265$, $df = 113$, $p = 0.791$). The only significant differences were for the Locomotor (PGD significantly lower) and Hearing and Language subscale (PGD significantly higher).

5.2.8 Child and Family Relationships

1. Parental questionnaires to assess family functioning

Overall there were 5 sets of missing questionnaires giving a response rate of 96%. This reflects a good response rate in returning questionnaires. The questionnaires were sent to the parents at least a week prior to the appointment and collected from the parents at the appointment. Thus this proved a practical and reliable method of collecting questionnaires. Recent studies by Edwards and colleagues report that contacting participants before sending questionnaires and follow up contact significantly increased response rate (Edwards *et al.*. 2002;Edwards *et al.*, 2007). Golombok has previously hypothesised that where questionnaires are used, it is possible that IVF families may wish to portray a more positive picture in order to 'prove' that they are good parents (Golombok, MacCallum, & Goodman 2001). The high response rate from both the groups may be a 'courtesy' effect: the parents of PGD children willing to cooperate because of the medical assistance with their conception (van, Naaktgeboren, & Trimbos-Kemper 1996)and the parents of control group willing because their child a thorough paediatric assessment.

2. Analysis

Statistical analyses were conducted using SPSS for Windows 15.0. Between group differences on continuous variables were analysed using t-tests and means and standard deviations are reported. Chi-square tests were used for categorical

variables and frequencies and percentages are reported. Where continuous variables were expected to be highly correlated, such as separate subscales, multivariate analysis of variance (MANOVA) was used and the between–groups effects were reported. MANOVA was used to for the analysis of the subscales of the Griffiths Scale, the Parenting Stress Index, the Parental Acceptance-Rejection Questionnaire, and the Toddler Temperament Questionnaire. The omnibus test was used to determine if there were overall effects, and between-subjects tests were used to identify those variables where differences were.

3. Child socio emotional behaviour

There was no between group differences in the temperamental characteristics perceived by mothers. These findings were contrary to one of the initial hypotheses of the study but consistent with an earlier major European study (Barnes *et al.*. 2004). Barnes *et al.* reported that parents of children born using ART techniques reported their children as having similar temperaments and similar levels of behaviour problems to the naturally conceived group. It has been suggested in earlier studies that parents of assisted conception children have more intense relationships with their child and are more over protective (Golombok, MacCallum, & Goodman 2001;McWhinnie 1996) and this in turn leads to the differences in childhood temperament but the present findings support Barnes *et al.* and the probability that this may not be so.

Golombok and MacCallum (2003) (Golombok & MacCallum 2003) state that in relation to socioemotional development, assisted reproduction children appeared to be functioning well. The greater difficulties of IVF infants were based on maternal reports and probably resulted from the higher anxiety levels of IVF mothers. Studies during the pre-school and school-age years generally did not indicate a higher incidence of emotional or behavioural problems among assisted reproduction children.

Thus, overall differences in parental adjustment following infertility management do not appear to translate into differences in child psychosocial function (Gibson & McMahon 2004).

However, there are few studies that have included children at adolescence or beyond, and little is known about the consequences of conception by assisted reproduction from the perspective of the individuals concerned. Moreover, the existing studies are of variable quality

4. Parental well being and family functioning

No significant differences were found on the three Parental Stress Index subscales and total scores. There was also no difference in the total GHQ scores. Thus so far contrary to our hypotheses parents seem to cope with the extra stresses of PGD without evidence of ill effects on there well being and relationships.

As assisted reproductive technologies continue to evolve, so too will the challenges confronting families and practitioners. It is inevitable that some families will pioneer the way in dealing with new issues in parenting and relatedness as they arise. In conclusion, despite the fact that these families have "complex biogenetic origins (Burns 1999), their family relationships are more like those of naturally conceiving families than not.

5. Parent –child relationship

Overall, the existing literature is reassuring for infertile couples who are contemplating assisted reproductive techniques, as well as for parents and health professionals: the psychosocial functioning of parents and their children born by assisted reproduction is more similar than dissimilar to that of the control families. In most cases, no statistically significant differences in child functioning in terms of emotions, behaviour, self-esteem, or perceptions of family relationship have been found. Parents of children born using assisted reproduction tend to report less parenting stress and more positive parent-child relationships than the control parents.

Gibson & McMohan (2004) (Gibson & McMahon 2004)reported that childhood parental expressions of more protective attitudes, but not over protectiveness towards the child, and greater warmth. The evidence supported the notion that protective social factors (e.g. older, educated, financially stable) and parental

qualities (e.g. satisfying relationships, adaptive problem solving, desire to have a child) may buffer against adverse parental outcomes.

Creating families by means of assisted reproduction has raised a number of concerns about potentially adverse consequences for parenting and child development. It seems, however, from the evidence available so far, that such concerns are unfounded. Parents of children conceived by assisted reproduction appear to have good relationships with their children

5.2.9 Strengths of the study

1. Participation rate

An initial estimate of at least 100 children less than eight years from the four PGD centres, who could be potentially recruited for the study, was made. Out of an original 100 qualifying children only 70 living within the London Region were eligible for the study. Of these 70 a total of 49 (70% participation rate) children were seen. This is a positive participation rate and could have been better if recruitment was extended to other national centres. However, owing to practical constraints this could not be done. In previous National studies of other children born after precious pregnancies, participation rates of 90% have been reported. This could be due to the fact that the mothers are likely to be highly motivated, given their good fortune to have been able to bear children (Barnes *et al.*. 2004;Sutcliffe *et al.*. 2001).

2. Comparison groups

Two groups were chosen, children born after PGD and a naturally conceived control group. The control group provided a standard of 'normal' by which to compare the PGD children.

3. Outcome measures

The developmental assessment used in this study was Griffiths Mental Development Scales from birth to 2 years (1996 Revision) and the Griffiths Mental Development Scales from birth to eight years (1984). The Griffiths Mental Development Scales from birth to 2 years were re-standardised in the United Kingdom in 1996 to reflect the child rearing practices, social habits, racial groupings and other socio-demographic factors pertinent to modern day Britain. The extended 2-8 years Griffiths scales were undergoing the most recent update and re-standardising for a fairly long time. There were delays in publication of the updated scales and thus it was not used in this study. This may have exaggerated the neurodevelopmental scores of the older children.

4. Single and blinded paediatrician

The blinding of the paediatrician to the mode of conception reduced observer bias and added to the strength of the study. A separate researcher approaching families for recruitment achieved blinding. The informed consent process also involved asking families not to reveal their conception status to the paediatrician. Analyses were also done by the statistician (MS) blind to the conception status. In addition all the children were assessed by the same paediatrician (IB), providing consistency in assessments and negating the inter observer bias.

5. Personal contact and advice lines

After the assessments the parents had a chance to discuss any queries or concerns with the paediatrician. The parents appreciated this and they were reassured. It is worth noting that families who participated in this preliminary study expressed gratitude and were grateful that their child had been formally assessed indicating an unfulfilled medical need, which may lead to an improved participation rate for later and larger studies. Recruitment is always a challenge in research of this nature.

6. The nature of the study

This preliminary study is the first detailed study published on children beyond the newborn period following PGD and provides provisional reassurance that these children do not have any overt problems over and above those known to be a risk from ART conception. This preliminary study showed that the methodology is suitable to assess a larger group of children.

7. Lessons learnt from PORD study that helped the PGD study

The difficulties encountered in the initial study (PORD) helped a lot with the PGD study. The recruitment was extended to multiple London fertility centres that obviously increased the particioation rate. The initial invitations to participate in the PGD study were sent from the respective fertility centres. The parents were much likely to respond to this, as they knew the fertility centres very well. The case and control recruitmet was also carried out by research assistants leading to the blinding of the assessor (IB).

5.2.10 Weaknesses of the study

1. Recruitment

The recruitment of IVF children was limited by UK law. The Human Fertilisation and Embryology Act 1990 doe not allow the HFEA or fertility clinics to disclose information about any individual who may have been conceived after assisted conception. Therefore any study in the UK must recruit using an 'opt- in' method. We asked clinics to send letters of invitation to families on our behalf, but we only became aware of these families if they chose to respond to us and disclose their details. We were therefore unable to obtain data on those families that did not respond and this could be a possible source of bias within our study.

2. Missing cases

An attempt was made to collect information from all families who had been recruited, but who did not attend. However, in a proportion of cases, families were either uncontactable due to change of address or contact details or the family declined to participate. Due to ethical constraints we were not able to ascertain

causes of non participation. Families who had shown interest initially were often unable to attend due to the distance they had to travel; the family sometimes didn't attend mutually arranged appointments, mother giving birth to new baby in family, ill health of mother and/or child etc.

3. Sample size

The sample size was small and was not adequate to obtain statistically reliable outcomes for many of the outcomes measured. Although the study was not adequately powered for definite conclusions, it nevertheless provided reassuring data that there was no evidence in the children born after PGD of major health concerns, effects on the mother child relationship or neurodevelopment scoring following PGD.

4. Growth data

The children were assessed at different ages and thus there was the variability of difference of age at assessment. This could have been overcome by a common statistical way of standardizing data on one scale so a comparison could take place. One way to achieve this was to use z-score so that the variability of assessment at different age could be overcome.

Chapter 6

Summary of thesis findings - main conclusions from the PORD and PGD/S studies.

6.1 PORD study

The results of this study were generally reassuring for the families where the mother have chronic renal disease and have had children. The study had aimed to address the following hypotheses:

Hypothesis 1 Children born to mothers with chronic renal disease are expected to have the following complications in comparison to age matched children born to well mothers:

- *Reduced longitudinal growth - refuted*
- *Possible effects on neurodevelopment - refuted*

Study and control children were comparable for growth parameters and neurodevelopmental scores as assessed by the Griffiths scales of mental development.

Numbers were small. However the data does provide reassurance to a group of mothers with a variety of renal disease that there was no effect related to maternal disease or medications used on growth and development of the children.

- *Greater occurrence of difficult temperament and emotional or behavioural problems – not refuted*

The study highlights significant differences in externalising behaviour (e.g. rule breaking and aggressive behaviour) between the study and the control groups. The numbers involved were small and further studies would be needed to establish this. The result might relate to the comparative social disadvantage (as assessed by the social class classification) seen in a higher proportion of PORD mothers than control mothers.

Hypothesis 2 Families where children are born to mothers with chronic renal disease will experience the following

- *Differences in maternal bonding.*
- *More stress in the parent child relationship.*
- *Other difficulties in parenting (related to pressures from renal disease and its treatment).*

There was no difference in the temperamental characteristics perceived by mothers in study and control groups. There was no evidence of more stress amongst mothers with renal disease or evidence of impaired bonding between mother and child in comparison with controls.

The hypothesis was refuted by the findings of the study. There have been concerns about psychological health of women with chronic renal disease and also how it might affect parenting. The current data is reassuring but numbers were small. So further studies are needed.

Hypothesis 3

- *The severity of effects will be directly related to the severity (stage) of chronic renal failure.*
- *Different effects will be observed in children of renal transplant recipients.*

The number of cases in the study was insignificant to provide strong evidence about the relation of severity of renal failure and outcome of the children. This was further compounded by difficulties in gathering maternal data from case notes.

Even though there were only eight mothers post transplant and the study provided some preliminary data to suggest that the wellbeing of these children were comparable with that of children born to well mothers. However further larger studies are needed in the future.

6.2 PGD study

This study is the first detailed study of children born after PGD world wide who were over a year of age and provides provisional reassurance that these children are healthy in comparison to naturally conceived children. The study aimed to address the following hypotheses:

Hypothesis 1 Children born to couples who had PGD/PGS are expected to be as healthy when compared to age matched naturally conceived children. We did not expect to see any of the following effects:

- *Reduced longitudinal growth*
- *Greater occurrence of difficult temperament and emotional or behavioural problems*
- *Effects on neurodevelopment*.

Growth parameters and neurodevelopmental scores were comparable in the study and the control group, providing reassuring information for couples who have undergone the procedure and also future couples who will be undergoing the procedure. The children studied did not show any temperamental, behavioural or emotional difficulties. Thus the hypothesis was confirmed within the limited number of cases in this study. Further larger studies are required in future.

Hypothesis 2 Families where children are born post PGD/PGS however may experience the following:

- *Differences in maternal bonding.*
- *More stress in parent child relationship.*
- *Other difficulties in parenting (related to the pressures of IVF, PGD/PGS and treatment).*

The PGD group had significantly higher scores on the warmth-affection sub-scale, and significantly lower scores on the aggression-hostility and rejection sub-scales than the control group. There was also no indication of increased levels of stress related to parenting.

The findings were contrary to the hypotheses but consistent with an earlier major European study which showed that ART families had higher levels of warmth and lower levels of hostility towards their children (Barnes *et al.,* 2004). Thus so far contrary to our expectations parents seem to cope with the extra stresses of PGD/S without evidence of ill effects on these relationships.

6.3 Conclusion

The studies and literature reviews in this thesis are generally reassuring, in terms of physical, neurodevelopmental and behavioural health of children born to mothers who encountered interventions in early life.

The studies also show evidence of generally positive relationships in families who are thought to be under considerable stresses from either chronic illness in the mother (chronic renal disease) or the stresses of infertility.

However, there remain areas of concern. The PORD study families participating with renal disease were more likely to be from lower socio-economic backgrounds. A significantly lower rate of vaginal deliveries was reported for mothers with renal disease and their infants were more likely to experience neonatal morbidity. The PGD study reported that 'PGD' children did not have any overt risk of problems over and above those known to be associated with ART. Children born after ART require to remain under continued follow up in view of the already known and discussed risks in chapter 2

Our study groups comprised two different groups of mothers but both the groups had one very striking similarity: the children had been exposed to interventions very early on in life and there were concerns about the consequences of these interventions.

Normal development may be disrupted by early adverse environmental influences; individuals who survive may have to cope with potentially damaging consequences. Additionally, the effects of environmental challenges in early life and the responses required to deal with them may have long-term effects into adulthood (Gluckman *et al.*. 2005)Barker *et al.* have clearly shown that the quality of fetal life can have profound effects on future well being of offspring (Barker *et al.*. 2002;Barker 2004;Poulter 2001).

Preimplantation genetic diagnosis is now an established alternative to prenatal diagnosis for couples carrying genetic conditions that may affect their offspring. In recent years PGD technology has been used increasingly in couples undergoing fertility treatment mainly to screen for aneuploidy (PGS). By doing this (PGS) it is expected that a variety of IVF outcomes can be improved. However there is conflicting evidence about the efficacy of PGS and its role in improving outcomes of fertility treatment. The ESHRE PGD consortium data collection VII (Harper *et*

al.. 2008) reporting on cycles up until Dec 2004 reported a steady increase in the number of cycles (PGD, PGS etc), of pregnancies and of babies delivered. It is expected that this rise will continue.

An anonymous editorial published in *Lancet* in 1975 noted "Children of women with renal disease used to be born dangerously or not at all — not at all if their doctors had their way." Women in various stages of renal failure were discouraged from conceiving because of concerns regarding the wellbeing of the off spring and also the wellbeing of the mother. These ranged from effects of maternal disease on the pregnancy and the fetus, effects of the medications (used to treat the mother), on the outcome of off spring, to effects of the pregnancy on the course of disease progression, effects on graft function/dysfunction and graft rejection. The author(s) in Lancet in 1975 were probably not prepared to speculate on the emerging era of fertility drugs, assisted reproduction techniques, and improvements in renal replacement therapy.

Subsequent progress in management of end-stage renal disease, ultimately by means of renal transplantation, has resulted in a lot more women living their lives into child bearing years. Having a child is now accepted as a set target of therapy success, major outcome of of quality of life and more and more women are going ahead with planning and having a family.

This increasing number of pregnancies may result in increasing problems related to the mother and fetus being exposed to interventions of different and changing tpyes. Interventions could result from the consequences of maternal health, disease management or infertility. True reassurance of the safety of these technologies will only be achieved by following a large cohort of these children throughout their lives. Findings of the present studies and the areas of concern highlighted should be taken into consideration by those undertaking resaerch in similar areas.

6.4 Areas for future research

1. Long term follow up of children born after PGD/PGS

This preliminary study is the first detailed study published on children born following PGD/PGS beyond the newborn period and provides provisional reassurance that these children do not have any overt problems. The physical health of children and normal developmental and behavioural outcomes was

reassuring, but future follow up is warranted. The mean age at assessment was 18.4 months (range 3- 56 months). It is possible that other problems (health, developmental and behavioural) may emerge as the children become older and can only be assessed by future studies preferably longitudinal. Development of a child is best assessed over a period of time rather than a one-off encounter. This study cohort should be followed up to detect any emerging difficulties.

The analyses did not distinguish between PGD for known genetic problems and PGD for aneuploidy screening (PGS) due to the small sample size. A larger study with adequate power for sub group analysis would be worthwhile. Another refinement although problematic from a control recruitment perspective would be to match for maternal age in a larger study.

2. Follow up of children born to mothers with chronic renal disease

This unique but modest study showed early evidence of wellbeing in mature children born to mothers with renal disease in pregnancy. However it also showed evidence of increased perinatal risks. A larger registry based study would seem one of two ways to confirm the key findings. The registry based study would also provide a chance to study children of mothers with different categories of renal impairment. Alternatively, the issue could be investigated via a prospective cohort study where further evidence of health status can be obtained. It seems important to carry out studies with larger number of cases to establish any cause–effect relation between severity of renal failure and outcome of the children. The effects of medications (especially new generation immunosuppressants) on the developing fetus and the children need to be investigated in detail.

Finally…

Those involved in providing and regulating treatment to families, be it for fertility associated problem or chronic renal disease, have an obligation to the patients, their children and families to ensure that any risk associated with the procedures are elucidated and the families informed. Many couples seeking infertility treatment are desperate for a child and similarly women with chronic renal disease often want to have a child. These individuals may not be deterred by the knowledge of a small increased risk in certain conditions, but they should have the

opportunity to be given accurate and robust information. More importantly, the children have a right to know of any potential risks that their future may hold.

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