INTRODUCTION

It is well known that environmental factors, particularly intrauterine nutrition, act in early life to program future cardiovascular and metabolic diseases and premature death. David Barker made epidemiological observations that established a link between birth weight, adult blood pressure and death from cardiovascular disease suggesting that environmental influences during foetal life play a major role in this association. This formed the basis of the “foetal origins of adult disease” hypothesis that was put forward by David Barker and colleagues in Southampton, United Kingdom. During the 1980s, David Barker exchanged these ideas with Nick Hales who suspected that the developing pancreatic beta cells in the foetus would be vulnerable to nutrient status during intra-uterine life and that this might predispose the offspring to development of diabetes in adulthood. He reasoned that the grouping of metabolic diseases (diabetes, ischemic heart disease and hypertension) could be explained on the basis of poor foetal nutrition. Through collaboration with David Barker on a cohort of 64-year-old men living in Hertfordshire for whom birth weight records were available, they demonstrated that men with a low birth weight were six times more likely to have diabetes than those with the highest birth weight. These findings formed the basis of the “Thrifty Phenotype Hypothesis” which stated that
“when the foetal environment is poor, there is an adaptive response, which optimizes the growth of key body organs to the detriment of others and is designed to enhance postnatal survival under conditions of poor nutrition” and were put forward by Nick Hales during his Banting Award Lecture in Dublin in 1991\(^5\). This was an extension of the “Thrifty Genotype Hypothesis” put forward by Neel in 1932\(^6\). He had hypothesized that during evolution, when food resources were not readily available or during times of famine, “thrifty genes” were selected. According to Neel, this resulted in a “fast insulin trigger” leading to increased storage of fat especially visceral fat. This lead to a heightened risk of diabetes mellitus and cardiovascular disease.

Before David Barker proposed the foetal origins hypothesis, associations between early life events and later cardiovascular diseases had been reported. Kermack et al in 1934 showed that death rates in the United Kingdom and Sweden were reduced between 1751 and 1930 and this was attributed to better living conditions\(^7\). Forsdahl carried out studies in Norway that showed the association between infant mortality rates in 1894-1897 and coronary heart disease in later life in 1964-1967. He hypothesized that “poverty may act through a nutritional deficit to result in a life-long vulnerability to a more affluent adult life-style.”\(^8\) Wadsworth, in 1985 showed an inverse relationship between
adult blood pressure and birth weight in both men and women born in 1946. 9

A variety of epidemiological studies carried out after Barker extended the observations of the relation between birth weight and adult disease. These studies found associations between growth patterns after birth and increased risks for obesity, hypertension, dyslipidemia, cardiovascular disease, inflammatory states, insulin resistance and diabetes mellitus in adult life.10 Thus the intrauterine experiences mould the foetal systems and this is called as “programming” that influences future health.11,12,13,14 The foetal origin hypothesis was thus extended to include all these components of the metabolic syndrome and is now known as the “Developmental Origins of Health and Disease (DOHaD). Few studies have, however, reported the associations between intrauterine undernutrition and reproductive health.

Molecular biological and evolutionary ecological studies have shown that a given genotype can give rise to multiple different type of phenotypes based on the surrounding environment11. These adaptations in the species can impact the development and behaviour of future species and can persist across many generations.11, 12 These have been referred to as epigenetic changes and refer to “covalent modifications of DNA & core
histones that regulate gene activity without altering the nucleotide sequence of DNA.” ¹

Peter Gluckman proposed to separate the foetal adaptations to undernutrition that may have long term advantages from those homeostatic responses that do not provide an immediate advantage but are in response to the future adaptive changes that the adult may have to face. He labelled these later responses as “predictive adaptive responses” and he further showed that such responses that provide an immediate advantage may not be appropriate in the adult life. He then labelled these responses as “inappropriate adaptive responses.” ¹³,¹⁴

However, all of these adaptations may not be predictive of the post natal environment especially when the nutritional deprivation may be severe. This results in structural and functional loss of nephrons, cardiomyocytes and pancreatic β cells. This results in a life-long reduction of functional capacity and is usually a response to allow growth of important organs like the brain, the brain sparing effect. The mechanism through which these changes are observed due to a reduced or inappropriate nutritional environment in foetal life include epigenetic changes, altered cell numbers, apoptosis and endocrine signalling or hormonal imprinting.¹

Epidemiological studies have tried to provide evidence for at least some of these observations. An increased incidence of death from stroke and
cardiovascular death was observed in a cohort of men and women who were born in Hertfordshire, England between 1911 and 1930.\textsuperscript{15} This was attributed to the lower birth weights and weights at 1 year in this population. The Swedish cohort of 15000 men and women with a follow up of 50 years showed that death rates due to ischemic heart disease (IHD) were the highest in individuals who were in the lowest quartiles of birth weights.\textsuperscript{16} Another Swedish study reported increased diastolic pressures in male army recruits who were born small for gestational age (SGA) and by implication had an increased risk for death from cardiovascular disease.\textsuperscript{17}

Yajnik et al in their studies in the Pune Maternal Nutrition Study (PMNS) have shown the presence of the “Thin-Fat” Indian phenotype that has a lower body weight, is centrally obese, are insulin and leptin resistant, develop diabetes a decade earlier and show compromised soft tissue growth as compared to the Caucasian (White) population. This phenotype is present right from birth and is a heightened risk for development of the Insulin Resistance (Metabolic) Syndrome. Similar cohorts have been studied in other places in India like Mysore, Delhi etc. However Yajnik et al were the first to show the “Thin-Fat” phenotype in the Indian Population.\textsuperscript{38}
Animal studies have given an insight in the role of foetal undernutrition in the physiological adaptations due to the nutritional insult. This causes permanent systemic changes that result in pathological consequences in the adult. Persson and Jansson ligated one uterine horn in guinea pigs to cause foetal growth restriction. This resulted in hypertension in the pups restricted for growth\textsuperscript{18}. Langley and Jackson showed that pregnant rats who were fed a low protein diet lead to hypertension in the pups after weaning at 4 weeks. Increased peripheral resistance was the cause but no cardiac hypertrophy was observed\textsuperscript{19}.

The kidney and the renin-angiotensin system are the primary targets of programming leading to hypertension. Brenner proposed that a reduction in nephron number and consequent sodium retention is seen in intra-uterine growth restriction leading to hypertension\textsuperscript{20,21}. It was later shown that single nephron GFR increased due to the reduced nephron number. A sustained increase in this pressure leads to focal glomerulosclerosis causing further nephron loss setting up of a vicious cycle causing irreversible renal damage\textsuperscript{22, 23, 24}. In growth restricted pregnant ewes, renin and angiotension mRNAs were significantly reduced\textsuperscript{25}. The renin angiotensin system (RAS) is known to play a very important role in the development of kidney\textsuperscript{26}. A decreased stimulation by the RAS (due to a reduction in mRNA) would probably lead to a decreased nephrogenesis.
It has been demonstrated in a model of uteroplacental insufficiency, induced by bilateral uterine ligation of the pregnant rats, that there was a significant reduction in the glomeruli number in full term foetal kidneys. Similar studies showing an up to 30% reduction in the nephron number have been reported in rabbits & pigs. The decrease in nephron number was associated with a parallel drop in GFR. Maternal protein restriction throughout pregnancy has also been shown to produce a significant deficit in nephron number, a decrease in kidney function & increased sodium concentration in the offspring in early postnatal & adult life.

Chronic hypoxia during gestation in rats has been shown to cause apoptosis and increased size and percentage of bi-nucleated myocytes in the heart. A 50% nutrient restriction in the pregnant sheep have been shown to have caused a change in gene expression that have been implicated in cardiac hypertrophy or cardiac remodeling.

A decrease in oxidative phosphorylation has been observed in growth restricted rats. An uncoupling of hepatic cellular energy and redox states leading to decreased ATP synthesis has also been observed in these rats. Concurrently, in growth restricted rats defects have been observed in mitochondria of the muscles leading to decreased supply of energy from ATP by oxidative phosphorylation. A decrease in ATP synthesis leads to
reduced recruitment of GLUT-4 to the surface of the cell, glucose transport and glycogen synthesis.\textsuperscript{36}

The effects of poor foetal nutrition on pancreatic development using a variety of approaches have shown a decrease in foetal pancreatic endocrine tissue, β cell mass, insulin concentrations, pancreatic & islet cell number & pancreatic insulin content.\textsuperscript{37,38,39} It has also been observed that there is either a decrease in proliferation & increase in apoptosis or decrease in cellular neogenesis.\textsuperscript{40,41} A decrease in vasculature of the pancreas & a decrease in insulin response of the foetal pancreas to Arginine & Taurine have been observed.\textsuperscript{42,43} During postnatal development a decrease in β cell to 50% of controls at 15 weeks & by 26 weeks of age a reduction to one third of normal has been observed.\textsuperscript{44}

Nutrition is known to affect the reproductive success in a variety of animal species. Under-nutrition is known to result in loss of body weight, delay the onset of puberty, increase the postpartum interval to conception, and interfere with the normal ovarian cyclicity by decreasing the gonadotropin secretion & increase infertility. In cows it has been observed that restricting energy intake during late gestation is known to increase the length of postpartum anoestrous & reduce subsequent pregnancy rate. It is also known to cause delayed uterine involution, delayed first oestrous after calving & increased incidence of cystic
Maternal undernutrition in sheep indicate that meiotic maturation of germ cells is delayed by food restriction.\textsuperscript{47}

It has been shown that maternal undernutrition in rats significantly reduced primordial, secondary & antral follicle number & mRNA levels of regulatory genes. These changes have been thought to be mediated by increased oxidative stress coupled with decreased ability to repair the resultant oxidative damage.\textsuperscript{48}

In beef cows undernutrition reduced plasma progesterone levels, number of ovarian follicles, altered the length of oestrous cycle & reduced the proportion of heifers with normal fertilized ova.\textsuperscript{49}

Epidemiological studies have shown that women who were exposed to the Dutch famine in 1944-45 showed greater reproductive success.\textsuperscript{50} Similar studies by Anita Ravelli et al showed that women exposed to the Dutch famine in Amsterdam were centrally obese and had a higher body mass index (BMI).\textsuperscript{51}

Barker’s hypothesis of the foetal origins of adult disease has now been proved by many epidemiological studies that have looked into the association between undernutrition and its effect on the endocrine system, cardiovascular disease and diabetes mellitus. Even though metabolic syndrome and development of cardiovascular disease and diabetes mellitus are important in current population and can be explained on the
basis of the foetal origins of adult disease, changes in the reproductive function as indicated by early puberty, increased incidence of Polycystic Ovarian Syndrome, infertility and early menopause need to be considered in relation to the Barker’s hypothesis. The animal models investigating the role of undernutrition on the reproductive system are based on short term, single nutrient restriction (for example, protein) either in utero or in the later life. This has little semblance to human undernutrition in the developing countries like India, which usually affects all aspects of nutrition (macro and micro nutrients) and is usually prolonged, sometimes multigenerational. There is a need for an animal model that will reflect human undernutrition more closely. A recent study on undernourished rats (Thrifty Jerry) carried out by Hardikar et al tried to closely mimic these conditions in the humans. These rats were given an undernourished diet that constituted 50% of the daily nutrient intake of adult (8 week old) Wistar rats for 50 generations. These Thrifty Jerry rats were born small, had a lower birth weight, were insulin resistant, were centrally obese but soft tissue growth (muscle, liver etc) was compromised, were deficient in Vitamin B\textsubscript{12} and folate and had higher homocysteine levels. They showed altered epigenetic signatures especially with the insulin -2 gene promoter region and these changes were unaltered even when these rats were restored to a normal diet. These
changes may thus be responsible for the altered metabolic profile and may signify similar changes in the human population. The present study has tried to evaluate the association between foetal undernutrition and reproductive function in the undernourished group of rats (Thrifty Jerry model) and an undernourished group of rats that was given a standard diet (The Recuperation or Transition model) to understand the systemic and epigenetic changes of 50 generations of undernutrition on the female reproductive system.