REVIEW ARTICLE (A Narrative Review)

THE CHANGING FACE OF MEDICINE IN THE 21ST CENTURY – THE EXAMPLE OF INFERTILE CLOMID FAILED INSULIN RESISTANT POLYCYSTIC OVARIAN PATIENTS SUBMITTED TO ASSISTED REPRODUCTIVE THERAPY (ART) IN PAKISTAN

Sarwat Jehan, Peter Baillie*

ABSTRACT

The outcome of metformin administration in insulin resistant infertile polycystic ovary syndrome (PCOS) patients who had failed clomid. The research was conducted at the University Assisted Reproductive Techniques (ART) Institute in Pakistan – a developing country with a high prevalence of diabetes. 166 PCOS patients submitted to ART out of 277 referrals (59.9%). Of these 78 (47%) achieved pregnancies with 75.6% take home babies and 24.4% reproductive failure prior to ART, Intra Cytoplasmic Sperm Injection (ICSI) and In Vitro Fertilization (IVF)). The total healthy take home baby rate was 47% of the 166 patients. These seventy eight (78) patients became pregnant prior to ART after starting metformin for insulin resistance. 88 required ART and 19 of these delivered a healthy baby. Metformin (500 to 2500 mg daily) was started prior to pregnancy and continued throughout pregnancy. Metformin 1500 mg daily for 3 months resulted in 59 live and well neonates averaging 3.1kg. Metformin <1500 mg daily and / or administration for less than 3 months prior to Pregnancy resulted in 18 abortions and 1 intrauterine death at 30 weeks gestation with no live neonate. Almost 50% became pregnant without ART. When ART was needed 19 out of 88 patients delivered a live baby. Almost 50% of patients with failed ART elsewhere became pregnant on this regime with take home healthy babies and 6 did not require ART. Metformin 1500mg daily administrated for at least 3 months prior to pregnancy and continued throughout pregnancy had an uncomplicated neonatal outcome in 59 patients compared to lower doses or shorter duration of administration in 19 patients who had a uniformly poor pregnancy outcome. The European Society of Human Reproduction and Embryology (ESHRE) and American Society for Reproductive Society (ASRM) guidelines are biologically and statistically flawed. In South Asia infertile PCOS patients require a long term life cycle approach. Early insulin resistance should be evaluated and metformin is the drug of choice in our hands. This has major implications for the life cycle of the patient, particularly as type II diabetes is now considered to be reversible.

Keywords: Metformin, PCOS, insulin resistance (IR), clomid, pregnancy outcome, life cycle, reversibility of type II diabetes.

1. INTRODUCTION

Clinical medicine in the 20th century was derived from the 1910 Flexner approach. Following a science based training, a clinical consultation window utilized history examination and pathological confirmation to conclude a diagnosis and administer treatment. The 21st century has developed this baseline extensively. Personalized, individualized patient care is applied using extensive information and based upon evidence, not expert guidelines.\(^1\) More importantly the life cycle of the patient needs to be considered together with the environment and particularly the community to apply prevention.\(^2\) The new WHO guidelines to decrease the dominant non infectious diseasesand deaths by 25% before 2025 demonstrates this.\(^3,5\)

Patients with polycystic ovaries presenting with
Infertility do not simply require infertility management, particularly because, since 1998 it has been clear that patients with polycystic ovaries presenting with symptoms such as infertility often have a metabolic disorder with a profound effect on the life cycle, particularly in Pakistan.

This communication examines the life cycle implications and short term pregnancy outcome in clomid failed insulin resistant infertile patients with polycystic ovaries presenting for assisted reproductive techniques such as Assisted Reproductive Techniques (ART), Intra Cytoplasmic Sperm Injection (ICSI) and In Vitro Fertilization (IVF) at a University Clinic in Pakistan.

The success of management and outcome will be described and the implications considered. The hypothesis to be tested is that insulin resistance should always be excluded in infertile South Asian patients with polycystic ovary syndrome (PCOS) and that, for these patients, metformin is the treatment of choice with early diagnosis of insulin resistance and attaining a favorable outcome. The life cycle approach in the 21st century favors gynecological infertility as a window of opportunity for prevention of later life cycle disease as well as improving pregnancy rates and outcome because it treats the cause. Metformin in doses of 1500 mg for at least 3 months prior to ART in patients with insulin resistance PCOS is hypothesized to treat the fetus effectively.

2. MATERIAL AND METHODS
Between January 2007 and December 2009, 277 patients with infertility and polycystic ovaries, who had failed to conceive after at least 3 cycles of clomid were referred for management by IVF or ICSI. After prolonged counseling, and exclusion of viral diseases (HIV, Hepatitis B and C) informed consent was signed. Contributory comorbidities of stress, weight, inflammation (including parainflammation tuberculosis and autoimmune disease) exogenous pharmaceutical agents and endocrinopathies (thyroid disease and insulin resistance in our patients) were minimized as well as any gynecological or general morbidity for 6 months prior to submission for ART.

Insulin resistance was diagnosed by a simplified MacAulay method as it includes a lipid component and diagnoses the condition prior to impaired glucose metabolism. Insulin resistance in PCOS patients was diagnosed when the serum insulin was >10 IU with triglycerides >150 mg% or >12 IU/ml without a raised fasting triglyceride level, using standard mainly Abbott Elisa Kits (normal range 2-27 miu/ml). Insulin resistance was found in 166 patients (59.9%) who were administered metformin in individualized doses ranging from 500 to 2500 mg daily, continued throughout any subsequent pregnancy. Eighty eight patients did not conceive while on metformin for 6 months and were submitted to ART with 19 (21.6%) mature, live and healthy neonates delivered. No abortions were recorded. In 78 patients who became pregnant prior to ART, hirsutism was present in 50 patients; acne was present in 28 patients and Luteinizing hormone (LH) > follicle-stimulating hormone (FSH) in 42 patients. Prior failed IVF cycles elsewhere were present in 30 patients.

Duration of infertility >6 yrs in 31 patients.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number</th>
</tr>
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<tbody>
<tr>
<td>15–30 yrs</td>
<td>41</td>
</tr>
<tr>
<td>31–35 yrs</td>
<td>25</td>
</tr>
<tr>
<td>&gt;35 yrs</td>
<td>10</td>
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</tbody>
</table>

* Two patients had inadequately recorded age.

<table>
<thead>
<tr>
<th>BMI</th>
<th>Number</th>
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<tbody>
<tr>
<td>20–25</td>
<td>35</td>
</tr>
<tr>
<td>25–30</td>
<td>23</td>
</tr>
<tr>
<td>&gt;30</td>
<td>10</td>
</tr>
</tbody>
</table>

Two patients had hypertension and 50 husbands were considered subfertile by WHO criteria.
3. RESULTS
3.1. ART After 6 Months Metformin
Eighty-eight patients with 19 mature healthy neonates resulted. No perinatal mortality and no abortions were recorded. Mild ovarian hyperstimulation occurred in 2 patients, both of whom had some residual polycystic ovarian changes after down regulation. Bromocriptine was used prophylactically.

No patients (n=19) of Baqai University Hospital that failed IVF and went elsewhere have become pregnant to our knowledge over 3 years. Conversely of 35 patients (30 with polycystic ovaries) that failed ART elsewhere were subsequently treated at Baqai Hospital, 17 became pregnant, six of whom did not require IVF or ICSI.

3.2. Pregnancy Prior to IVF on Metformin
The age and body mass index (BMI) outcome of pregnancies prior to IVF on metformin are reported in Table 1 and 2. Duration and dose of metformin prior to pregnancy and outcome are reported in Table 3. No maternal hypertension, diabetes or hypoglycemia or other complications occurred in the mothers and fetuses apart from the single intrauterine fetal death.

Neonatal condition – all 59 delivered at >37 weeks and had no neonatal complications. The average neonatal weight was 3.1 kg with a range of 2.5 to 3.7 kg. Follow up for normality at 3 years was carried out telephonically.

### Table 1. Age and outcome of pregnancies prior to IVF on metformin.

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Successful pregnancy</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–30</td>
<td>32</td>
<td>8</td>
</tr>
<tr>
<td>31–35</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>&gt;35</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

* P for trend < 0.05, Younger patients had a more successful outcome.

### Table 2. BMI and outcome of pregnancies prior to IVF on metformin.

<table>
<thead>
<tr>
<th>BMI</th>
<th>Successful pregnancy</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–25</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>25–30</td>
<td>17</td>
<td>6</td>
</tr>
<tr>
<td>&gt;30</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

### Table 3. Duration and dose of metformin prior to pregnancy and outcome.

<table>
<thead>
<tr>
<th>Duration and dose of metformin</th>
<th>Successful pregnancy</th>
<th>Abortion</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1500 mg for &gt;3 months</td>
<td>33</td>
<td>2</td>
</tr>
<tr>
<td>&lt;1500 mg or &gt;1500 mg for &lt;3 months</td>
<td>17</td>
<td>6</td>
</tr>
</tbody>
</table>

1 IUD at 30 weeks was a gestational diabetic who stopped metformin at ±7 weeks gestation.

No overlap is present, so statistics are not necessary.

4. DISCUSSION
Diabetes in Pakistan\(^9\) and now in China\(^10\) has a very high incidence in adult life with multiple co-morbidities. These become apparent later in the life cycle but clearly should be considered in acute management. The major precursor of type II diabetes is the metabolic syndrome first described by Geoffrey Reaven\(^11\) who considered insulin resistance, which varies in different organs, to be the fundamental pathological cause starting with a compensatory hyperinsulinaemia\(^12,13\). Diabetes itself is considered to be due to widespread intracellular lipotoxicity\(^14\) which ultimately leads to failure of glucose homeostasis as excess carbohydrate intake is converted to lipids for storage. Translational medicine has shown that insulin producing β cells do not undergo apoptosis under this assault but simply dedifferentiate\(^15-17\). This makes type II diabetes potentially reversible with a very low carbohydrate intake\(^18\) and stimulation of β cells (incretins at present) and gastro intestinal surgery – the simple sleeve gastrectomy\(^19\) now being widely available in Pakistan.

The early management of the precursor metabolic syndrome and diabetes (as prevention of later disease in the life cycle is the aim in the 21st century) is
generally agreed to be lifestyle alteration and metformin therapy\textsuperscript{20,21}.

Polycystic ovaries are also a manifestation of the precursor metabolic syndrome with many other causes leading to a failure of progression of the ovarian cycle beyond the antral stage. In contrast to the agreed management in all other areas, gynecological management of polycystic ovaries and infertility favors treating (in the window of disease presentation to the gynecologist) the mechanism with clomid\textsuperscript{22-25} rather than the life cycle disease process with metformin if insulin resistance is present despite John Nestler’s statement in 1998 that there is often a serious causal underlying metabolic disorder\textsuperscript{6}.

This apparent anomaly has been brought about by the consensus European Society of Human Reproduction and Embryology (ESHRE)\textsuperscript{26} definition to allow adequate comparisons of what was an unsatisfactory chaos. The multiple causality has been reflected by a subdivision of androgenic polycystic ovaries by Roberto Aziz\textsuperscript{27} as they have an identifiable differing life cycle and phenotype.

Recent major reviews of the management of infertility with polycystic ovaries strongly support clomid although only one includes measurement of insulin resistance\textsuperscript{25}.

These reviews are largely centered on Europe and North America where obesity appears to be the dominant factor\textsuperscript{28,29}. A further problem is that the favored double blind epidemiological approach to management is inappropriate in syndromes with differing proportions of etiological factors\textsuperscript{30-33}. In South Asia\textsuperscript{34} and recently in China\textsuperscript{10} the incidence of diabetes and its consequences is high, conforming to the genomic intrauterine programming in deprivation of the Barker hypothesis,\textsuperscript{35-37} which differs in a major way from Europe and North American, even in emigrants to the developed world\textsuperscript{38,39} and also reflected in a different spectrum of polycystic ovarian disease in South Asia,\textsuperscript{34} a major component of which is the genomic intrauterine programming of the Barker hypothesis\textsuperscript{1} as compared with the developed world which is mainly obesity engendered\textsuperscript{25,24,40}, although obesity produces an additive double disease lead in Pakistan\textsuperscript{41}.

This study demonstrates that almost 50\% of patients with clomid failed insulin resistant PCOD associated infertility do not require ART if metformin in doses of at least 1500 mg is started at least 3 months before pregnancy in order to achieve a successful outcome. This conforms to the basic science that meiotic development of the ovum to the antral stage, which is also androgen dependant,\textsuperscript{42} takes some 200\textsuperscript{43,44} days and explains why fetal abnormalities are due to high glucose levels during ovum development\textsuperscript{45,46} and not metformin\textsuperscript{47}. Insulin like growth factor II and citrate levels are low in the granulosa cells of insulin resistant patients\textsuperscript{48,49} thereby possibly explaining the higher dose of metformin required if the well recognized early reproductive failure by abortion is to be avoided\textsuperscript{50}.

The almost uniformly successful outcome in our patients is encouraging and suggest the hypothesis that metformin in insulin resistant PCOD patients produces better ovulation, implantation placentation and fetal outcome than insulin which is indirect\textsuperscript{51,52} – there were no abortions, macrosomia or premature labour\textsuperscript{53}, neonatal condition was uncomplicated and development up to 3 years after birth appeared to be normal as childhood outcome has been reported to be suboptimal follows ART\textsuperscript{54}. This applied even to failed ART at other units presenting at BIRDS.

It is acknowledged that other interfering factors that are associated with infertility were treated\textsuperscript{55} – stress, weight, inflammation, including parainflammation and autoimmunity, exogenous substances and hormonal diseases (not mechanisms).
The lack of concurrence with meta analysis of ESHRE randomized double blind trials is simple. A syndrome will lead to confusing and sometimes incorrect outcomes if differing proportions of cause are included. There is good evidence that South Asians have a different form of PCOS related to the Barker hypothesis probably stemming from partition and driven by genomic expression of intrauterine programming including insulin resistance to account for the much higher incidence of later onset diabetes and its consequences in South Asia (and recently in China where the much lower BMI and different incidence of diabetes in deprivation suggests that the very high incidence has a component of the Chinese disruption and famine in 1959–61). This holds true even for emigrants to the west, who still, because of birth conditions, have a doubled mortality and morbidity. In contrast, the worldwide obesity epidemic leads to a milder form of insulin resistance, which may well be clomid responsive in the meta analyses and as an additive factor in deprivation leading further to insulin resistance. This proliferation of confusing randomized studies in a syndrome is incorrect mathematically, which is why we chose to study the predominant insulin dependent form in South Asia rather than a diffuse multifactorial presentation. This has clarified our approach. It should be realized that metformin is not a silver bullet cure all, as 88 patients still required ART with the expected outcome apart from no pregnancy failure when we added metformin throughout pregnancy. Nevertheless metformin treatment of gestational diabetes does lead to satisfactory outcomes with decreased abortions although none targeted ovum normality by prepregnancy use as we did. The effect of metformin is modest and via the canonical ART pathway. Inisotol acting upstream at the cell border also stimulates the oncogenic cMyC and RAS pathways, so is not suitable for long term use but may be helpful in pregnancy. It should be noted that we used the more sensitive MacAuley method in young patients as the HOMA IR is mainly used in older patients with failing glucose control in order to predict diabetes in the near future.

There have been recent advances in understanding the development of PCOD in clinical practice in the life cycle. Pubertal acne is due to PCOD development temporarily due to IR produced by growth hormone. If it lasts more than 2 years it probably has an underlying component. More importantly the pathogenesis of type II diabetes in the life cycle suggests strongly that insulin resistance with infertility and PCOD needs to have lifelong preventative intervention rather than simply treating a window of infertility as is practiced at present.

The most understandable pathogenesis is that diabetes probably starts with insulin resistance due to widespread intracellular lipotoxicity (usually secondary to dietary overload and intrauterine genome programming in Pakistan, China and India) which ultimately resulting in homeostatic failure and hyperglycaemia. The β cells do not undergo atrophy but dedifferentiate to mesoderm under this metabolic and nutritional assault, suggesting that early diabetes, at least, can be reversed with a very low carbohydrate diet and β cell simulation (probably incretins at present) as well as bariatric surgery with the simple sleeve gastrectomy widely available in Pakistan.

Insulin administration in type II diabetes has negative effects as it is a growth hormone and metformin has been shown decrease and delay conversion of precursors to diabetes, result in a lower incidence of heart attacks and their sequelae and limitation of metastatic breast cancer as well as many other conditions including Alzheimer’s disease, endometrial cancer as well as mortality limitation and fibroids. To this may be added prevention of the link to adult disease suggested by the Barker hypothesis through conception and early pregnancy normalization as we found. This makes it clear that polycystic ovarian disease is not a gynaecological disease but a systemic condition with major
significance for the life cycle requiring early diagnosis and continued treatment after pregnancy and a major opportunity in the prevention for reproductive biologists if patients present with insulin resistance.\textsuperscript{75,76}

This analysis must be viewed as a translationally informed hypothesis in the 21st century provoking further study in Pakistan rather than acceptance of western epidemiological guidelines,\textsuperscript{77} as it is an observational cohort from a single institution in a developing country of a highly selected group of infertility patients. The conclusions must be tentative and replication is required.\textsuperscript{78,79}

In conclusion, we are encouraged by the outcome reported in what must be considered as a proof of principle in the 21st century approach of life cycle analysis and input to infertile PCOD patients in Pakistan.

5. ADDENDUM
As insulin resistance must have a similar incidence in subfertile males although with a different endocrine abnormality. Prof. Mehmood Yousef carried out a pilot study on 8 subfertile males selected by IDF criteria starting with waist / height >50%. Of 8 patients given metformin, 6 significantly improved their semen analysis and 4 produced normal pregnancies within 1 year.

6. ACKNOWLEDGEMENTS
The biologist and laboratory technicians at BIRDS are an intrinsic critical team in obtaining successful outcomes. Prof. Mrs. Zahida Baqai, the Vice Chancellor for her initiative in instigating BIRDS in a developing country as well as her never failing input.

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