Openventio PUBLISHERS

ISSN 2377-1542

GYNECOLOGY AND OBSTETRICS RESEARCH

Open Journal

| July 2016 | Volume 3 | Issue 1|

www.openventio.org

GYNECOLOGY AND OBSTETRICS RESEARCH

Openventio PUBLISHERS

ISSN 2377-1542

Open Journal

TABLE OF CONTENTS

GYNECOLOGY AND OBSTETRICS RESEARCH

Openventio PUBLISHERS

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-127

Mini Review

Corresponding author
Qiwei Yang, PhD
Senior Research Scientist
Division of Translation Research
Department of Obstetrics and Gynecology
Augusta University
Medical College of Georgia
Augusta, GA, USA
Tel. 706-721-8801
E-mail: QYANG@augusta.edu

Volume 3 : Issue 1
Article Ref. #: 1000GOROJ3127

Article History
Received: February 18th, 2016
Accepted: May 2nd, 2016
Published: May 3rd, 2016

Citation
Prusinski L, Al-Hendy A, Yang Q. Developmental exposure to endocrine disrupting chemicals alters the epigenome: Identification of reprogrammed targets. *Gynecol Obstet Res Open J.* 2016; 3(1): 1-6. doi: 10.17140/GOROJ-3-127

Developmental Exposure to Endocrine Disrupting Chemicals Alters the Epigenome: Identification of Reprogrammed Targets

Lauren Prusinski; Ayman Al-Hendy, MD, PhD; Qiwei Yang, PhD*

Division of Translation Research, Department of Obstetrics and Gynecology, Augusta University, Medical College of Georgia, Augusta, GA, USA

ABSTRACT

Endocrine disruptions induced by environmental toxicants have placed an immense burden on society to properly diagnose, treat and attempt to alleviate symptoms and disease. Environmental exposures during critical periods of development can permanently reprogram normal physiological responses, thereby increasing susceptibility to disease later in life – a process known as developmental reprogramming. During development, organogenesis and tissue differentiation occur through a continuous series of tightly regulated and precisely-timed molecular, biochemical and cellular events. Humans may encounter Endocrine Disrupting Chemicals (EDCs) daily and during all stages of life, from conception and fetal development through adulthood and senescence. Though puberty and perimenopausal periods may be affected by endocrine disruption due to hormonal effects, prenatal and early postnatal windows are most critical for proper development due to rapid changes in system growth. Developmental reprogramming is shown to be caused by alterations in the epigenome. Development is the time when epigenetic programs are 'installed' on the genome by 'writers', such as histone methyltransferases (HMTs) and DNA methyltransferases (DNMTs), which add methyl groups to lysine and arginine residues on histone tails and to CpG sites in DNA, respectively. A number of environmental compounds, referred to as Estrogenic Endocrine Disruptors (EEDs), are able to bind to Estrogen Receptors (ERs) and interfere with the normal cellular development in target tissues including the prostate and uterus. These EEDs, including diethylstilbestrol (DES), bisphenol A (BPA), and genistein (a phytoestrogen derived from soybeans), have been implicated in the malformation of reproductive organs and later development of disease. Due to the lack of fully understanding the underlying mechanisms of how environmental toxicants and their level of exposure affect the human genome, it can be challenging to create clear clinical guidance to address the potential health effects of lower-level exposures commonly experienced within the general population. In addition, human studies concerning environmental exposures are limited in feasibility by ethical concerns for human safety. Therefore, studies in animal models provide great opportunities to reveal links between early-life exposure to EDCs and related diseases. It has been shown that developmental exposure to EDCs, such as diethylstilbestrol (DES) and genistein, during reproductive tract development increases the incidence, multiplicity and overall size of uterine fibroids in the Eker rat model, concomitantly reprogramming estrogen-responsive gene expression. Importantly, EDC exposure represses enhancer of zeste 2 (EZH2) and reduces levels of the histone 3 lysine 27 trimethylation (H3K27me3) repressive mark through Estrogen receptor/Phosphatidylinositide 3-kinases/Protein kinase B non-genomic signaling in the developing uterus. More recent research identified a developmental reprogramming target, *Scbg2a1* gene, whose epigenetic status can be altered by early exposure to BPA in the rat prostate. Molecular analyses revealed markedly increased expression (greater than 100 fold) of *Scgb2a1*, a secretaglobin gene in response to developmental exposure to BPA. This increase in *Scgb2a1* expression is concomitantly associated with increased enrichment of acetylated H3K9 (H3K9Ac representing active chromatin status) and hypomethylation of DNA for a CpG island upstream of the transcription start site of *Scgb2a1*. These data suggest that expression of *Scgb2a1* in the adult prostate could be epigenetically reprogrammed by BPA exposure during prostate development. Further studies are needed to create more targeted preventative interventions as well as specific, effective therapeutics to decrease the incidence of diseases.

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-127

KEYWORDS: Developmental environmental exposure; Endocrine disrupting chemicals; Epigenetics; Prostate; Fibroids.

ENDOCRINE DISRUPTION AND DISEASES

Endocrine disruption induced by environmental toxicants have placed an immense burden on society to properly diagnose, treat, and attempt to alleviate symptoms and disease.[1,2] Though not yet evaluated in the United States, a recent European study reports that the provision of long-term care and treatment for those affected by health conditions in which endocrine disrupting chemicals (EDCs) are suspected exceeds more than (the equivalent of) two billion dollars per year.[3] Environmental exposures during critical periods of development can permanently reprogram normal physiological responses, thereby increasing susceptibility to disease later in life a process known as developmental reprogramming. During development, organogenesis and tissue differentiation occur through a continuous series of tightly-regulated and precisely-timed molecular, biochemical and cellular events. Humans may encounter EDCs daily and during all stages of life, from conception and fetal development through adulthood and senescence. Though puberty and perimenopausal periods may be affected by endocrine disruption due to hormonal effects, prenatal and early postnatal windows are most critical for proper development due to rapid changes in system growth.[4] Additionally, EDCs differ from other environmental toxicants and chemicals in that the effects of EDCs are often induced at small doses and vary based on the window of time of exposure. Thus, these seemingly minor levels of exposure exert subtle changes at the molecular and cellular levels that ultimately induce more severe pathophysiologic effects. Exposure to these and other environmental chemicals has been linked with infertility, delayed puberty, and premature birth,[5] as well as with later development of several diseases such as diabetes mellitus,[6] cardiovascular disease,[7] and particularly neoplasia.[8,9] Notably, early developmental exposures contribute to trans-generational inheritance of phenotype.[10]

DEVELOPMENTAL REPROGRAMMING AND EPIGENETIC REGULATION

It has been well-established that the genetic makeup of a human being, i.e. the human genome, plays a major role in determining predisposition to developing certain diseases. Family medical histories, for example, may demonstrate trends of increased risk of breast cancer due to family members carrying the *BRCA1/2* gene mutation.[11] Yet another example includes parent carriers of a mutation in the *CFTR* gene offspring from these carriers are more likely to be diagnosed with cystic fibrosis as compared to parents who do not carry a *CFTR* mutation.[12] Classical twin designs can decompose genetic and environmental sources of variance. More difficult to elucidate, however, is how a person's physical environment alters the expression of his or her genome. The molecular mechanisms as to how exposure to external variables, e.g. diet, exposure to chemicals or radiation, climate, or medications, ultimately affect the regulation of the human epigenome leading to increased risk of developing disease are not well understood.

In recent years, more research has focused on the effects these environmental exposures have on a developing fetus. During fetal development, the human genome's expression can be adapted to suit proper development of tissues in response to the fluctuating needs of the growing fetus' body genetic expression is altered to maintain physiologic conditions that optimize the fetus' chance for survival and continued growth. Unfortunately, the plasticity of genetic expression during this critical time in fetal development can also negatively impact the developing fetus as the fetus responds to adverse stimuli. A historical example of this was evidenced in infants born to mothers prenatally administered thalidomide: infants exhibited limb malformation; or diethylstilbestrol (DES): girls developed clear cell carcinoma of the vagina later in life.[3,14]

Developmental reprogramming is shown to be caused by alterations in the epigenome.[2,13,14] Epigenetic modifications play an important role in 'programming' lineage determination and cellular identity during development.[15] Several different types of epigenetic modifications are thought to contribute to the alteration of gene expression during development.[16,19] Among many type of epigenetic proteins which play a role in epigenetic modification, histone methyltransferases (HMTs) and DNA methyltransferases (DNMTs) function as epigenetic "writers", which add methyl groups to lysine and arginine residues on histone tails and to CpG sites in DNA, respectively.[20,21] For histone modifications, the epigenetic programs that are installed by these writers form a 'histone code' that is interpreted by 'readers' (effector molecules that recognize histone modifications) and modified by 'erasers' such as histone demethylases.[20,22,23] Methylated CPG sites are also remodeled during this time *via* Ten-eleven translocation (TET) enzymes that function as erasers for DNA methylation, converting 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) and other oxidation products.[24,25] The activity of epigenetic enzymes can be altered through specific post-translational modifications (PTMs) leading to activating or inactivating enzymes, or regulating modifier binding of chromatin indirectly. For example, phosphorylation, one of the common PTMs, occurs *via* kinase mediators, e.g. cyclin-dependent kinases, (CDKs), protein kinase A (PKA), and protein kinase B (PKB/Akt) on these "readers, writers, and erasers" of histone methyl marks. The specific histone marks can be correlated with the activity of specific effector proteins, methyltransferases, and demethylases which ultimately play a role in epigenome reprogramming.[2,26,27] The epigenetic modifiers whose activities are altered *via* phosphorylation include enhancer of Zeste homolog 2 (EZH2), mixed-lineage leukemia protein 1 (MLL1), and lysine-specific histone demethylase 1A (LSD1).[13] The activity of epigenetic enzymes is particularly important during development, because they play an important role in remodeling the epigenome after fertilization and during gametogenesis[24], as well as in several types of cancer.[28]

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-127

ENVIRONMENTAL EXPOSURE AND ESTROGEN SIGNALING

A number of environmental compounds referred to as estrogenic endocrine disruptors (EEDs), are able to bind to estrogen receptors (ERs) and interfere with the normal cellular development in target tissues including the prostate and uterus. These EEDs, including diethylstilbestrol (DES), bisphenol A (BPA), and genistein (a phytoestrogen derived from soybeans), have been implicated in the malformation of reproductive organs and later development of disease.[2]

These past mass-exposures to endocrine disrupting chemicals, like DES, during reproductive tract development have been linked with reprogramming of estrogen-responsive gene expression in the uterine myometrium. This leads to tissue hyper-responsiveness to ovarian sex hormones, specifically estrogen and progesterone, later in adult life, and predisposes women to the development of uterine leiomyoma.[27] Animal studies have shown that early-life exposure to DES during uterine development (in rats, uterine development occurs post-natally) permanently alters the morphology of the reproductive tract *via* an "estrogen imprint" despite the readily-metabolized nature of DES and its efficient clearing from the body.[3] These experiments have provided evidence of the long-term, permanent pathophysiological effects of even brief exposure to environmental endocrine disruptors, such as DES. The challenge remains, however, to connect exposure to other environmental toxicants which result in permanent epigenetic changes and disease-related outcomes.

DEVELOPMENTAL EXPOSURES TO EDCS INCREASE UTERINE FIBROID DEVELOPMENT

Due to the lack of fully understanding the underlying mechanisms of how environmental toxicants and their level of exposure affect the human genome, it can be challenging to create clear clinical guidance to address the potential health effects of lower-level exposures commonly experienced within the general population. In addition, human studies concerning environmental exposures are limited in feasibility by ethical concerns for human safety. Therefore, studies in animal models provide great opportunities to reveal links between early-life exposure to EDCs and related human diseases including uterine fibroids. For example, Eker ($Tsc^{Ek/+}$) rats, heterozygous for the tuberous sclerosis 2 (*Tsc2*) gene, i.e. carrying one *Tsc2* mutant allele, have a 65% incidence of spontaneously developing uterine fibroids, generally around 12 months of age.[8] Dr. Walker's group described the long-term effects of postnatal exposure to EDCs (DES, and genistein) on the uteri of Eker rats after allowing them to develop to 16 months of age.[14] Early-life exposure to EDCs including DES or genistein increased tumor penetrance (from 65% to >90%), tumor multiplicity, and overall size. While the molecular mechanisms are still being revealed, these experiments with genistein show that in the developing uterus, genistein induces epigenetic changes of non-genomic estrogen receptor (ER) signaling by way of activation of the PI3K/AKT pathway. This in turn phosphorylates histone methyltransferase Enhancer of Zeste Homolog 2 (EZH2), a potent epigenetic regulator of gene expression and inactivates EZH2. This ultimately reduces levels of H3K27me3 found in chromatin, which are normally a mark of repressed gene expression.[14] Thus, the overall expression of estrogen-responsive genes is, in turn, increased.[8] Even 16 months following brief exposure to genistein during uterine development, their studies showed increased activity of EZH2 suggesting interference with epigenetic programming in the development of the uterus, leading to permanent alterations that persist into adult life. Further investigation is needed to identify the direct epigenetic link between developmental programming targets and EDC exposures during uteri development.

DEVELOPMENTAL EXPOSURE TO BPA INCREASES THE RISK OF CARCINOGENESIS IN THE PROSTATE

Besides the uterus, the prostate is another altered organ targeted by adverse developmental exposure. Induction of carcinogenesis in the prostate in response to developmental exposure to BPA has been reported.[29] In the animal model, brief developmental exposure to BPA ultimately induces later estrogen-mediated carcinogenesis of the prostate in rats. Prins et al. conducted experiments using progenitor cells expressing estrogen receptors (ERs)-α and β, derived from prostate glands of young, disease-free men. These cells, when grafted into a kidney-capsule mouse model for tissue formation, formed normal human prostate epithelium that produced prostate-specific antigen (PSA). When these mice were treated with testosterone and estradiol (T+E), the prostate tissue began to show pathologic progression from normal tissue growth to hyperplasia and finally prostatic intraepithelial neo plasia over a 4-month period.[15] These findings suggest that the estrogen-responsiveness of prostate stem and progenitor cells may provide a link to the epigenetic disruption caused by early-life BPA exposure in the human prostate, potentially leading to carcinogenesis of the prostate.[29]

A recent study by Wong et al further supports the link between exposure to environmental toxicants during plastic developmental periods and epigenetic alteration of gene expression.[30] This study identified a developmental reprogramming target, *Scbg2a1* gene, whose epigenetic status can be altered by early exposure to BPA in the rat prostate. As depicted in the Figure 1, using an animal model of early developmental exposure, rat pups were exposed (either subcutaneously or orally, to mimic the route of exposure likely to occur in humans) to BPA at postnatal days 1, 3, and 5 in three separate populations. The following were analyzed: 1) serum BPA levels; 2) molecular changes in the prostate at 70 days post-BPA-treatment; 3) histopathologic changes in the prostate at 12 months after the rats were implanted with T+E-containing capsules at Day 70 to drive prostate carcinogenesis. The histopathologic results in the population maintained for the 12-month longitudinal demonstrated dysplasia (the rat equivalent of human prostatic intraepithelial neoplasia), with the incidence of dysplasia increasing with increasing oral dose of BPA. Additionally, both BPA-exposed and vehicle groups treated with T+E on and after 70 days demonstrated adenocarcinomas and carcinomas *in situ*, indicative of

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-127

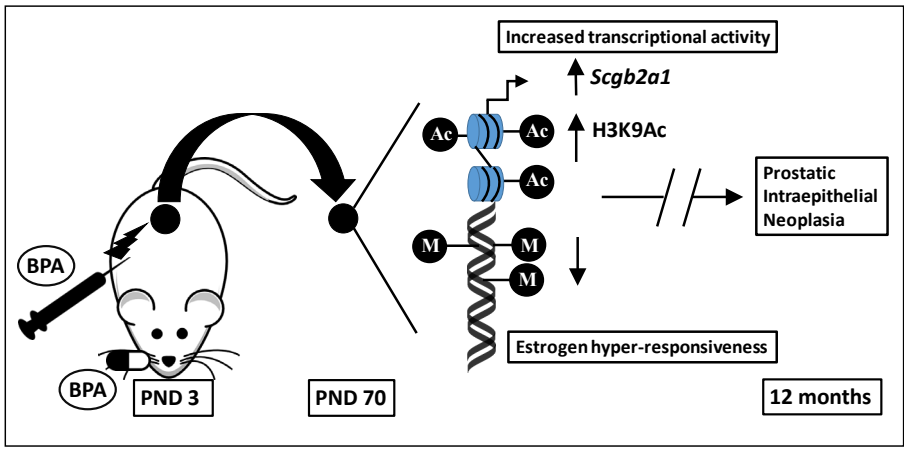

Figure 1. Early-life exposure to BPA results in increased enrichment of acetylated H3K9 (H3K9Ac representing active chromatin status) and hypomethylation of DNA for a CpG island upstream of the transcription start site of *Scgb2a1*.
M: Methyl; Ac: Acetyl; PND: postnatal days; BPA: Bisphenol A.

these rats' susceptibility to T+E-promoted carcinogenesis in the prostate.[30]

Molecular analyses revealed markedly increased expression (greater than 100 fold) of *Scgb2a1*, a secretaglobin gene in response to developmental exposure to BPA. This increase in *Scgb2a1* expression is concomitantly associated with increased enrichment of acetylated H3K9 (H3K9Ac representing active chromatin status) and hypomethylation of DNA for a CPG island upstream of the transcription start site of *Scgb2a1*. These data suggest that expression of *Scgb2a1* in the adult prostate could be epigenetically reprogrammed by BPA exposure during prostate development. Further potential implications include increased risk for cancer in response to chemotherapeutics associated with prostate in binding.[30] Though the functional significance of reprogrammed *Scgb2a1* has yet to be fully elucidated, there is evidence of its being a marker of carcinogenesis and disease recurrence in ovarian cancer. Its over expression has also been found in endometrial and lung cancers.[16] *Scgb2a1* has been proposed to be involved in micro-metastasis *via* the lymph node in abdominal cancers, biliary tract carcinoma, and breast cancer indicating its potential role as a gene that can be reprogrammed. It remains unclear, however, as to whether it serves as a marker of reprogramming rather than a driver of carcinogenesis.[16] Discovering genes like *Scgb2a1* and understanding the mechanisms connecting them to developmental reprogramming could manifest into libraries of potential biomarkers of epigenetic alterations. These biomarkers could help predict one's predisposition to developing disease and one's response to therapeutics as a result of early-life exposure to environmental toxicants, events that would otherwise seem disconnected by the span of time between them. It remains to be determined whether these effects of reprogramming are similarly present in the human prostate gland and whether similar early-life exposure to BPA can be determined to mediate later prostate carcinogenesis in humans.

CONCLUDING REMARKS

With increasing emphasis on the susceptibility and mechanisms of developmental environmental exposures, greater advances will be made toward identifying the biomarkers useful in predicting human predisposition to disease. Moreover, researchers can then focus on creating more targeted preventative interventions as well as specific, effective therapeutics to decrease the incidence of disease. Though the complexity of signaling pathways, gene expression patterns, and the vast array of environmental toxicants may blur the lines connecting early cause and late effect, each step towards increased knowledge will bring humanity closer to decreasing the burden on society that poor health sets upon it.

ACKNOWLEDGEMENT

This work was supported in part by an Augusta University Start-up package, the National Institutes of Health grant HD04622811 (to AA), and the Augusta University Intramural Grants Program (QY). We would like to thank Walidah Walker, MPH for editing this manuscript.

CONFLICTS OF INTEREST

None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

REFERENCES

1. Heindel JJ. Role of exposure to environmental chemicals in the developmental basis of reproductive disease and dysfunction. *Semin Reprod Med*. 2006; 24(3): 168-177. doi: 10.1055/s-2006-944423

2. Walker CL, Ho SM. Developmental reprogramming of cancer susceptibility. *Nat Rev Cancer*. 2012; 12(7): 479-486. doi: 10.1038/nrc3220

3. Trasande L, Zoeller RT, Hass U, et al. Estimating burden and disease costs of exposure to endocrine-disrupting chemicals in the European union. *J Clin Endocrinol Metab*. 2015; 100(4):1245-1255. doi: 10.1210/jc.2014-4324

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-127

4. Gore AC, Heindel JJ, Zoeller RT. Endocrine disruption for endocrinologists (and others). *Endocrinology*. 2006; 147(6 Suppl): S1-S3. doi: 10.1210/en.2005-1367

5. Crews D, McLachlan JA. Epigenetics, evolution, endocrine disruption, health, and disease. *Endocrinology*. 2006; 147(6 Suppl): S4-S10 doi: 10.1210/en.2005-1122

6. Chevalier N, Fenichel P. Endocrine disruptors: new players in the pathophysiology of type 2 diabetes? *Diabetes Metab*. 2015; 41(2): 107-115. doi: 10.1016/j.diabet.2014.09.005

7. Roseboom TJ. Undernutrition during fetal life and the risk of cardiovascular disease in adulthood. *Future cardiology*. 2012; 8(1): 5-7. doi: 10.2217/fca.11.86

8. Cook JD, Davis BJ, Cai SL, Barrett JC, Conti CJ, Walker CL. Interaction between genetic susceptibility and early-life environmental exposure determines tumor-suppressor-gene penetrance. *Proc Natl Acad Sci U S A*. 2005; 102(24): 8644-8649. doi: 10.1073/pnas.0503218102

9. Cook JDA, Davis BJ, Goewey JA, Berry TD, Walker CL. Identification of a sensitive period for developmental programming that increases risk for uterine leiomyoma in Eker rats. *Reprod Sci*. 2007; 14(2): 121-136. doi: 10.1177/1933719106298401

10. Bernal AJ, Jirtle RL. Epigenomic disruption: the effects of early developmental exposures. *Birth Defects Res A Clin Mol Teratol*. 2010; 88(10): 938-944. doi: 10.1002/bdra.20685

11. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. American journal of human genetics. 2003; 72(5):1117-1130. doi: 10.1086/375033

12. Heller K. Genetic counseling: DNA testing for the patient. *Proceedings*. 2005; 18(2):134-137. Web site. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1200714/. Accessed February 17, 2016.

13. Ly L, Chan D, Trasler JM. Developmental windows of susceptibility for epigenetic inheritance through the male germline. *Semin Cell Dev Biol*. 2015; 43: 96-105 doi: 10.1016/j.semcdb.2015.07.006

14. Sandovici I, Hammerle CM, Ozanne SE, Constancia M. Developmental and environmental epigenetic programming of the endocrine pancreas: consequences for type 2 diabetes. *Cell Mol Life Sci*. 2013; 70(9): 1575-1595. doi: 10.1007/s00018-013-1297-1

15. Cantone I, Fisher AG. Epigenetic programming and reprogramming during development. *Nature structural & molecular biology*. 2013; 20: 282-289 doi: 10.1038/nsmb.2489

16. Li H, Richardson WD. Genetics meets epigenetics: HDACs and Wnt signaling in myelin development and regeneration. *Nat Neurosci*. 2009; 12(7): 815-817. doi: 10.1038/nn0709-815

17. Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. *Clinical epigenetics*. 2015; 7:120 doi: 10.1186/s13148-015-0155-4

18. Rasmussen TP. The epigenetics of early development: inferences from stem cells. *Mol Reprod Dev*. 2014; 81(2): 194-201. doi: 10.1002/mrd.22269

19. Van de Vijver G, Van Speybroeck L, De Waele D. Epigenetics: a challenge for genetics, evolution, and development? *Ann N Y Acad Sci*. 2002; 981:1-6. doi: 10.1111/j.1749-6632.2002.tb04909.x

20. Trevino LS, Wang Q, Walker CL. Phosphorylation of epigenetic "readers, writers and erasers": Implications for developmental reprogramming and the epigenetic basis for health and disease. *Prog Biophys Mol Biol*. 2015; 118(1-2): 8-13 doi: 10.1016/j.pbiomolbio.2015.02.013

21. Dawson MA, Kouzarides T, Huntly BJ. Targeting epigenetic readers in cancer. *N Engl J Med*. 2012; 367(7): 647-657. doi: 10.1056/NEJMra1112635

22. Berdasco M, Esteller M. Aberrant epigenetic landscape in cancer: how cellular identity goes awry. *Dev Cell*. 2010; 19(5): 698-711. doi: 10.1016/j.devcel.2010.10.005

23. Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. *Nat Rev Mol Cell Biol*. 2007; 8(12): 983-994. doi: 10.1038/nrm2298

24. Tan L, Shi YG. Tet family proteins and 5-hydroxymethylcytosine in development and disease. *Development*. 2012; 139(11): 1895-1902. doi: 10.1242/dev.070771

25. Yang Q, Mas A, Diamond MP, Al-Hendy A. The Mechanism and Function of Epigenetics in Uterine Leiomyoma Development. *Reprod Sci*. 2015; 23(2): 163-175. doi: 10.1177/1933719115584449

26. Bredfeldt TG, Greathouse KL, Safe SH, Hung MC, Bedford MT, Walker CL. Xenoestrogen-induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT. *Mol Endocrinol*. 2010; 24(5): 993-1006. doi: 10.1210/me.2009-0438

27. Greathouse KL, Bredfeldt T, Everitt JI,et al. Environmental estrogens differentially engage the histone methyltransferase EZH2 to increase risk of uterine tumorigenesis. *Mol Cancer Res*.

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-127

2012; 10(4): 546-557. doi: 10.1158/1541-7786

28. Huang Y, Rao A. Connections between TET proteins and aberrant DNA modification in cancer. *Trends Genet*. 2014; 30(10): 464-474. doi: 10.1016/j.tig.2014.07.005

29. Prins GS, Hu WY, Shi GB, et al. Bisphenol A promotes human prostate stem-progenitor cell self-renewal and increases in vivo carcinogenesis in human prostate epithelium. *Endocrinology*. 2014; 155(3): 805-817. doi: 10.1210/en.2013-1955

30. Wong RL, Wang Q, Trevino LS, et al. Identification of secretaglobin Scgb2a1 as a target for developmental reprogramming by BPA in the rat prostate. *Epigenetics*. 2015; 10(2):127-134. doi: 10.1080/15592294.2015.1009768

GYNECOLOGY AND OBSTETRICS RESEARCH

Openventio PUBLISHERS

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-128

Research

*Corresponding author
Reham R. Elkhateeb, MD
Senior Lecturer of Obstetrics and Gynecology
Faculty of Medicine Minias University
Minia Maternity and Pediateric University Hospital, Minia, Al Minya, Egypt
Tel. 00201011966648
E-mail: rehamelkhateeb78@yahoo.com

Volume 3 : Issue 1
Article Ref. #: 1000GOROJ3128

Article History
Received: April 15th, 2016
Accepted: May 18th, 2016
Published: May 19th, 2016

Citation
Elkhateeb RR, Mahran A. Optimized letrozole dose versus traditional use of clomiphene citrate for ovulation induction in patients with PCOS: a prospective randomized controlled trial. *Gynecol Obstet Res Open J*. 2016; 3(1): 7-12. doi: 10.17140/GOROJ-3-128

Optimized Letrozole Dose *Versus* Traditional Use of Clomiphene Citrate for Ovulation Induction in Patients With PCOS: A Prospective Randomized Controlled Trial

Reham R. Elkhateeb, MD*; Ahmad Mahran, MRCOG, MD

Senior Lecturer of Obstetrics and Gynecology, Faculty of Medicine Minia University, Minia, Al Minya, Egypt

ABSTRACT

Objective: To compare the effects of gradually increased letrozole dose in *versus* Clomiphene Citrate (CC) (100 mg) for ovulation induction in women with polycystic ovary syndrome (PCOS).
Design: Prospective randomized controlled trial.
Setting: IVF unit at Minia Maternity University Hospital in Egypt.
Patient(s): Two hundred infertile women with PCOS defined according to Revised Rotterdam criteria.
Intervention(s): patients were randomly allocated into two groups; study group (100 patients) receiving gradually increased doses of letrozole starting with 2.5 mg on cycle day 3 with incremental increase of 2.5 mg daily till reaching a dose of 10 mg daily on cycle day 6 and a control group (100 patients) receiving CC at a dose of 100 mg daily for 5 days starting from cycle day 3. Patients were followed up for three treatment cycles. The primary outcome was clinical pregnancy rate and the secondary outcome was number of mature follicles, endometrial thickness, serum progesterone and time to reach a dominant follicle.
Result(s): The two groups were similar in the demographic features and baseline hormonal milieu. There was no significant difference between the two groups as regards the number of mature follicles and the time to reach mature follicles. Endometrial thickness on HCG day was significantly higher in the letrozole group as compared with CC group (10.1±0.22 mm *vs* 8.2±0.69 mm, p=0.01)). Serum progesterone was higher in letrozole group than in CC group (19.3±3.1 *vs* 15.3±2.2, p<0.01). Ovulation was achieved in165/242 cycles (68.2 %) in the letrozole group and 169/249 cycles (67.9 %) in the CC group which was not statistically significant. Clinical pregnancy rate was significantly higher in letrozole group in comparison with CC group (14.8 % *vs* 10.4 %, p<0.01)
Conclusion(s): Letrozole in gradually increased dose achieves higher clinical pregnancy rate as compared with the traditional dose of CC. Therefore, it can be used as a first-line treatment for ovulation induction in women with PCOS.

KEYWORDS: Polycystic ovary syndrome; Letrozole; Clomiphene citrate; Ovulation induction.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory disorder in young women. It can be manifested in a variety of clinical presentations. It is estimated that 55% to 75% of women with PCOS are infertile due to chronic anovulation.[1,2] Clomiphene Citrate (CC) is still the first line treatment for ovulation induction. However, there is a marked discrepancy between ovulation rate and pregnancy rate achieved with CC which can be attributed to the negative effect on the cervical mucus and endometrium.[3,4]

Letrozole is a type II a third-generation aromatase inhibitor that has been widely used in women with breast cancer.[5] It works through inhibiting the conversion of and rostenedione

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-128

and testosterone to estrogen in the ovary which leads to estrogen depletion that creates negative feedback signals to hypothalamic-pituitary axis (HPA), therefore, follicle-stimulating hormone (FSH) secretion increases, stimulating the development of ovarian follicles.[6-9]

The aim of the current study was to compare the effects of gradually increased dose of letrozole *versus* the traditional dose of CC for ovulation induction in in infertile women with PCOS.

PATIENTS AND METHODS

This study is a randomized controlled trial including 200 women with PCOS recruited from those attending the Fertility unit at Minia Maternity University Hospitalin Egyptin the period from January 2013 to January 2014. Ethical approval for the study was obtained from the local ethical committee of the department of Obstetrics and Gynecology. All the eligible women signed a written informed consent before inclusion in the study. .

Inclusion criteria of the study were: a) Age from 18 to 35 years b) diagnosis of PCOS according to revised Rotterdam criteria,[10] c) Patent fallopian tubes proved by hysterosalpingography (HSG), d) normal semen analysis of the male partner according to the modified criteria of the World Health Organization.[11] We excluded patients with: a) history of laparoscopic ovarian drilling, b) uterine cavity abnormalities, and c) combined factors of infertility.

RANDOMIZATION

Patients were randomly allocated using a computer-generated random table into two groups:

• Study (letrozole) group (n=100):in this group, patients received letrozole (Novartis Pharma Services, Basel, Switzerland) starting with a dose 2.5 mg on cycle day 3 with 2.5 mg incremental increase in the dose till reaching a dose of 10 mg on cycle day 6.

Control (CC) group (n=100): in this group, patients received CC (Hoechst Marion Roussel, ARE) at a dose of 100 mg daily for 5 days starting from cycle day 3. The study flow chart is shown in Figure 1.

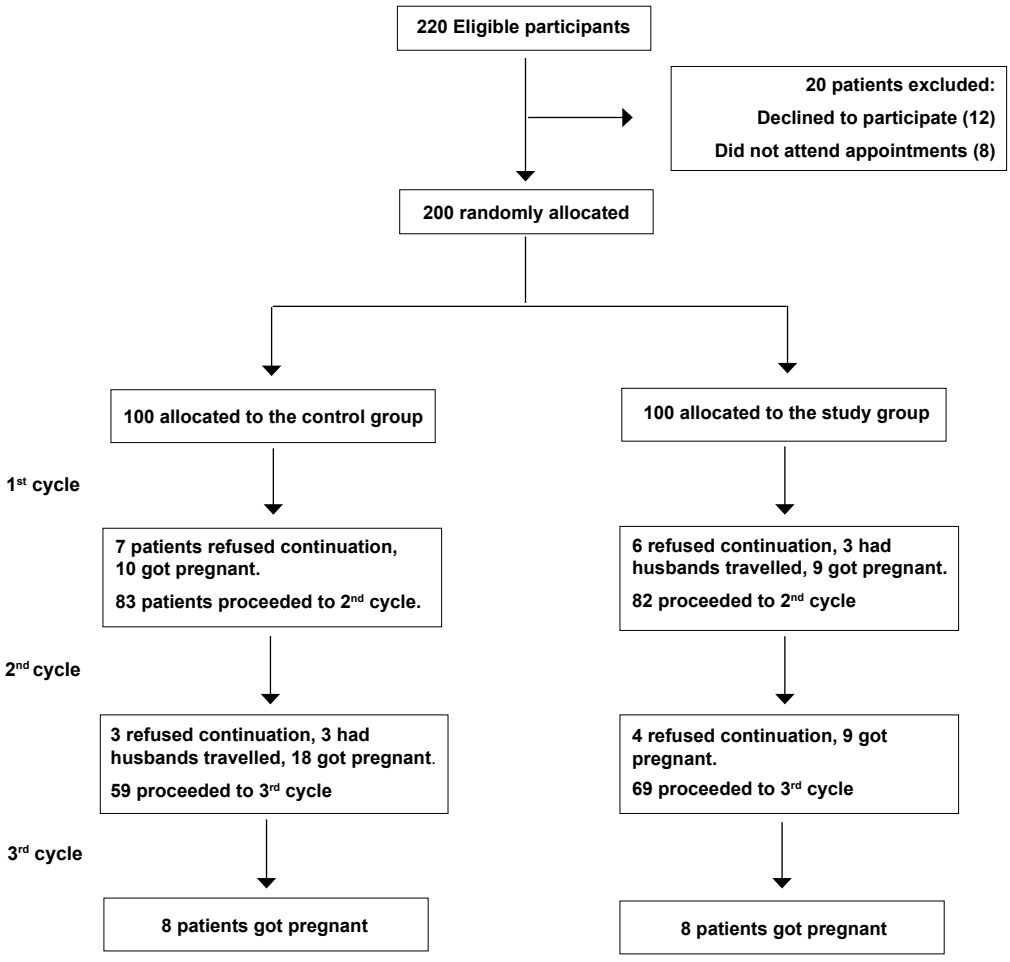

Figure 1: Study flow chart.

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-128

Patients were monitored with transvaginal ultrasound starting from the 6th day of stimulation and every other day till a dominant follicle reaching 18 mm in diameter. Human chorionic gonadotropin (hCG) injection (5000-10,000 IU IM) was commenced when at least one follicle measuring 18 mm was detected on ultrasound scan. Serum progesterone (ng/mL) concentration was measured on days 21 to 23 of the cycle by radioimmunoassay (RIA) using the antibody coated tube method (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA). Patients were advised to have intercourse 24 to 36 hours after the hCG injection. Serum pregnancy test was performed two weeks after the hCG injection. Clinical pregnancy was confirmed by detection of fetal pole and pulsation with ultrasound scan two weeks after a positive pregnancy test. If no pregnancy was achieved in the first cycle, the same treatment was given for a total of three treatment cycles.

OUTCOME MEASURES

• The primary outcome measure of the study was the clinical pregnancy rate.

• The secondary outcome measures were the number of mature follicles, endometrial thickness, serum progesterone and the time to reach a dominant follicle.

STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for Social Science (SPSS) version 17. Data were presented as mean and standard deviation (SD) for continuous variables, frequencies and percentages for categorical data. Chi square test was used to compare categorical data A $p<0.05$ % was considered statistically significant.

RESULTS

The study included 200 patients received 491 treatment cycles. There were no statistically significant differences between the two groups as regards the demographic features and hormonal milieu as shown in Table 1.

There was no significant difference between the two groups as regards the number of mature follicles and the time to reach mature follicles. Endometrial thickness on HCG day was significantly higher in the letrozole group as compared with CC group (10.1±0.22 mm *vs* 8.2±0.69 mm, $p=0.01$)). Serum progesterone was higher in letrozole group than in CC group (19.3±3.1 *vs* 15.3±2.2, $p<0.01$). Ovulation was achieved in 165/242 cycles (68.2 %) in the letrozole group and 169/249 cycles (67.9 %) in the CC group which was not statistically significant. Clinical pregnancy rate was significantly higher in letrozole group in comparison with CC group (14.8 % *vs* 10.4 %, $p<0.01$). There was one case of twin pregnancy in the CC group, but no ovarian hyperstimulation syndrome (OHSS) occurred in either group. (Table 2)

DISCUSSION

CC is most commonly used drug for ovulation induction in patients with PCOS. However, it has anti-estrogenic effect so it may be associated with poor cervical mucus and endometrial thinning due to prolonged estrogen-receptor depletion in the endometrium and possibly in the cervix; an effect that can explain the obvious discrepancy between the ovulation rate and the pregnancy rate achieved with CC.[3,4]

Letrozole; an aromatase inhibitor, has been tried by many researchers as an alternative treatment to CC in different

	Letrozole group (n=100)	CC group (n= 100)	*p* value
Age	24.8±3.1	25.3±2.9	0.67
Type of infertility:			
-Primary	67 (67 %)	71 (71 %)	0.74
-Secondary	33 (33 %)	29 (29 %)	
Duration of infertility(years)	4.1±3.1	5.1±2.2	0.81
BMI(kg/m2)	31.1±2.91	29.1±3.12	0.31
Clinical presentation			
Oligo/anovulation	95 (95 %)	92 (92 %)	0.75
Hyperandrogenism	44 (44 %)	42 (42 %)	0.68
Polycystic ovaries	85 (85 %)	70 (70 %)	0.08
FSH (IU/l)	6.1±2.92	6.3±2.2	0.63
LH (IU/L)	12.9±1.82	12.1±3.11	0.52
Testosterone(ng/ml)	0.62±0.3	0.61±0.2	0.64

Data is presented as mean±SD or number and percentage.

Table 1. Demographic features and hormonal profile of the study population.

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-128

	Letrozole group (n=100)	CC group (n=100)	*P* value
No. of stimulation cycle	242	249	0.83
No. of follicles (≥18 mm) on the day of hCG	3.4±0.5	3.8±0.6	0.04*
Endometrial thickness on the day of hCG	10.1±0.22	8.2±0.69	0.01*
Serum progesterone on day 21-23(ng/ml)	19.3±3.1	15.3 ± 2	<0.01*
Timeto reach a dominant follicle (days)	10.1±1.32	10.3±1.8	0.21
Clinical pregnancy rate per treatment cycle	36/242 (14.8 %)	26/249(10.4 %)	<0.01*
Multiple pregnancy	0	1	0.5
OHSS	0	0	0.93

Data is presented as mean±SD
*Statistically significant.

Table 2: Details of stimulation cycles and outcome measures in the study population.

regimens.[12-18] The optimal dose of letrozole for ovulation induction in patients with PCOS has not been yet determined. Most of the published studies had used letrozole in a fixed dose (2.5-7.5 mg) starting from cycle day 2 to 6. A novel step-up protocol of letrozole was used by Mitwally et al.[19] This study included 22 PCOS women in whom 9 women received letrozole in a step-up protocol consisting of one, two, three, and four tablets of letrozole (2.5 mg) daily on menstrual cycle days 2, 3, 4, and 5, respectively. The control group included 13 patients received 100 mg/day clomiphene citrate (CC) for 5 days starting on menstrual cycle day 3. The step-up letrozole protocol in that study was shown to achieve higher CPR per treatment cycle as compared with CC. The CPR per cycle reported by Mitwally et al was 27.3 % which is higher than the rate reported in the current study (14.8 %). The cause for this marked difference may be attributed to the use of intrauterine insemination in the first study while in the current study; patients were advised to have timed intercourse in addition to the relatively small number included in the first study.

Elham Rahmani et al used a step up protocol with serial increase in letrozole dose over three successive months of treatment. They started with 2.5 mg and the dose was increased according to response. They concluded that increasing the dose can improve the chance of ovulation and pregnancy.[20] However, we believe the protocol used in that study is consuming time and raises the cost of the treatment.

Mitwally and Casper[21] gave letrozoleat a fixed dose of 2.5 mg starting from cycle day 3 for 5 days in 12 patients with PCOS. Ovulation occurred in nine patients (75 %), and pregnancy was achieved inthree patients (25 %). In the current study letrozole was given with gradual increase of the dose started with 2.5 mg to reach 10mg to maximize the effect of letrozole in ovulation induction and try to decrease side effects (OHSS and multiple pregnancies) and at the same time reduce the cost of treatment per cycle. Clomiphene citrate results in central estrogen receptor depletion for a long duration because of its significantly greater half-time for clearance (2 weeks).[22,23] As a result, supra-physiological levels of estrogen can occur without central suppression of FSH because the normal estrogen receptor-mediated feedback mechanisms are blocked. This results in multiple follicle growth and in higher multiple pregnancy rates with CC than encountered with aromatase inhibitor cycles. Mitwally et al.[24] reported favorable pregnancy outcomes and a low multiple-gestation rate for the use of aromatase inhibitors for ovarian stimulation.

In the present study, CPR was higher in the letrozle group despite there was no significant difference in the number of mature follicles between the two groups. This can be explained by the adverse effect of CC on the endometrium and cervical mucus. This finding is not in agreement with Al-Fouzan et al who reported higher number of mature follicles in the letrozole group than in the CC group.[17] Endometrial thickness was significantly higher in letrozole group in the current study. Similar findings were reported by Mitwally and Casper.[21] Cortinez et al also reported normal morphologic features of endometrium and full expression of pinopodes during the implantation window when letrozole was used,[15] while Kilic et al and Bishai et al noted significant effect of both drug on the endometrium.[25,26]

Data about teratogenic capacity of letrozole in humans is lacking. Animal studies have shown that low doses of letrozole are effective in inducing noxious effects on the developing conceptus.[26] Large randomized controlled trials are required to evaluate the long term safety of letrozole use for induction and its optimal dose to balance between better pregnancy and neonatal outcome.

In conclusion, Letrozole in gradually increased dose is associated with higher CPR as compared with the traditional CC dose for ovulation induction in women with PCOS. Further studies are needed to confirm these findings and to provide stronger evidence for implication of such regimen in clinical practice.

CONFLICTS OF INTEREST: None.

REFERENCES

1. Kovacs G, Wood C. The current status of polycystic ovary syndrome. *Aust NZ J Obstet Gynecol*. 2001; 41(1): 65-68. doi: 10.1111/j.1479-828X.2001.tb01296.x

2. Slowey MJ. Polycystic ovary syndrome: new perspective on an old problem. *South Med J*. 2001; 94(1): 190-195. Web site.

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-128

http://europepmc.org/abstract/med/11235033. Accessed April 14, 2016

3. Thompson LA, Barratt CL, Thornton SJ, Bolton AE, Cooke ID. The effects of clomiphene citrate and cyclofenil on cervical mucus volume and receptivity over the periovulatory period. *Fertil Steril*. 1993; 59(1): 125-129. Web site. http://europepmc.org/abstract/med/8419199. Accessed April 14, 2016

4. Kousta E, White DM, Franks S. Modern use of clomiphene citrate in induction of ovulation. *Hum Reprod Update*. 1997; 3(4): 359-365. doi: 10.1093/humupd/3.4.359

5. Pfister CU, Martoni A, Zamagni C, Lelli G, De Braud F, Souppart C. Effect of age andsingle versus multiple doses, pharmacokinetic of letrozole (Femara) in breast cancerpatients. *Biopharm Drug Dispos*. 2001; 22(5): 191-197. doi: 10.1002/bdd.273

6. Naftolin F, MacLusky NJ. *Aromatization hypothesis revisited*. In: Serio M, ed. Differentiation: basic and clinical aspects. New York, USA: Raven Press; 1984: 79-91.

7. Naftolin F, MacLusky NJ, Leranth CZ, Sakamoto HS, Garcia-Segura LM. The cellular effects of estrogens on neuroendocrine tissues. *J Steroid Biochem*. 1988; 30(1-6): 195-207. doi: 10.1016/0022-4731(88)90093-3

8. Naftolin F. Brain aromatization of androgens. *J Reprod Med*. 1994; 39(4): 257-261. Web site. http://europepmc.org/abstract/med/8040841. Accessed April 14, 2016

9. Roberts V, Meunier H, Vaughan J, et al. Production and regulation of inhibin subunits in pituitary gonadotropes. *Endocrinology*. 1989; 124(1): 552-554. doi: 10.1210/endo-124-1-552

10. The Rptterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*. 2004; 19(1): 41-47. doi: 10.1093/humrep/deh098

11. World Health Organization. *WHO laboratory manual for the examination and processing of human semen*. 5th ed. Geneva, Switzerland: World Health Organization; 2010: 271.

12. Requena A, Herrero J, Landeras J, et al. Use of letrozole in assisted reproduction: a systematic review and meta-analysis. *Hum Reprod Update*. 2008; 14(6): 571-582. doi: 10.1093/humupd/dmn033

13. Begum MR, Begum A. Letrozolevs clomiphene citrate in induction of ovulation in polycystic ovarian disease (PCOD). *Fertil Steril*. 2006; 86(3): S408.

14. Mitwally MF, Casper RF. Single-dose administration of an aromatase inhibitor for ovarian stimulation. *Fertil Steril*. 2005; 83(1): 229-231. doi: 10.1016/j.fertnstert.2004.07.952

15. Cortinez A, De Carvalho I, Vantman D, Gabler F, Iniguez G, Vega M. Hormonal profile and endometrial morphology in letrozole-controlled ovarian hyperstimulation in ovulatory infertile patients. *Fertil Steril*. 2005; 83(1): 110-115. doi: 10.1016/j.fertnstert.2004.05.099

16. Holzer H, Casper R, Tulandi T. A new era in ovulation induction. *Fertil Steril*. 2006; 85(2): 277-284. doi: 10.1016/j.fertnstert.2005.05.078

17. Al-Fozan H, Al-Khadouri M, Tan SL, Tulandi T. A randomized trial of letrozole versus clomiphene citrate in women undergoing super ovulation. *Fertil Steril*. 2004; 82(6): 1561-1563. doi: 10.1016/j.fertnstert.2004.04.070

18. Tulandi T, DeCherney AH. Limiting access to letrozole-is it justified? *Fertil Steril*. 2007; 88(4): 779-780. doi: 10.1016/j.fertnstert.2007.01.115

19. Mitwally MF, T Said, A Galal, et al. Letrozole step-up protocol: a successful superovulation protocol. *fertil steril*. 2008; 89(4): S23-S24. doi: 10.1016/j.fertnstert.2008.02.071

20. Rahmani E, Ahmadi S, Motamed N, Maneshi HO. Dosage optimization for letrozole treatment in clomiphene-resistant patients with polycystic ovary syndrome: a prospective interventional study. *Obstet Gynecol Int*. 2012; 2012: 758508. doi: 10.1155/2012/758508

21. Mitwally M, Casper R. Use of aromatase inhibitor for ovulation induction in patientswith an inadequate response to clomiphene citrate. *Fertil Steril*. 2001; 75(2): 305-309. doi: 10.1016/S0015-0282(00)01705-2

22. Gonen Y, Casper RF. Sonographic determination of a possible adverse effect of clomiphene citrate on endometrial growth. *Hum Reprod*. 1990; 5(6): 670-674. Web site. http://humrep.oxfordjournals.org/content/5/6/670.long. Accessed April 14, 2016

23. Yagel S, Ben-Chetrit A, Anteby E, Zacut D, Hochner-Celnikier D, Ron M. The effect ofethinyl estradiol on endometrial thickness and uterine volume during ovulation induction by clomiphene citrate. *Fertil Steril*. 1992; 57(1): 33-36. Web site. http://europepmc.org/abstract/med/1730327. Accessed April 14, 2016

24. Mitwally M, Biljan M, Casper R. Pregnancy outcome after the use of an aromataseinhibitor for ovarian stimulation. *Am J Obstet Gynecol*. 2005; 192(2): 381-386. doi: 10.1016/j.ajog.2004.08.013

25. Kilic-Okman T, Kucuk M, Altaner S. Comparison of the effects of letrozole andclomiphene citrate on ovarian follicles, endometrium, and hormone levels in the rat. *Fertil Steril*. 2003; 80(6): 1330-1332. doi: 10.1016/j.fertnstert.2003.05.002

26. Bishai R, Arbour L, Lyons C, Koren G. Intrauterine exposure

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-128

to clomiphene andneonatal persistent hyperplastic primary vitreous. *Teratology*. 1999; 60(3): 143-145. doi: 10.1002/(SICI)1096-9926(199909)60:3<143::AID-TERA9>3.0.CO;2-#

GYNECOLOGY AND OBSTETRICS RESEARCH

Openventio PUBLISHERS

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-129

Case Report

*Corresponding author
Gabriel Carles, MD
Department of Obstetrics and Gynaecology, Saint-Laurent Hospital
97320 Saint-Laurent du Maroni
French Guiana
Tel. (594) 594348753
Fax: (594) 594348760
E-mail: g.carles@ch-ouestguyane.fr

Volume 3 : Issue 1
Article Ref. #: 1000GOROJ3129

Article History
Received: May 5th, 2016
Accepted: May 26th, 2016
Published: May 27th, 2016

Citation
Carles G, Dabiri C, Mchirgui A, et al. Different uses of chitosan for treating serious obstetric hemorrhages. *Gynecol Obstet Res Open J*. 2016; 3(1): 13-15. doi: 10.17140/GOROJ-3-129

Different Uses of Chitosan for Treating Serious Obstetric Hemorrhages

Gabriel Carles, MD*; Clement Dabiri, MD; Ali Mchirgui, MD; El Oula Saoudi, MD; Najeh Hcini, MD; Beatrice Seve, MD; Bruno de Matteis, MD

Department of Obstetrics and Gynecology, Saint-Laurent Hospital, 97320 Saint-Laurent du Maroni, French Guiana

ABSTRACT

Postpartum hemorrhage is a major cause of maternal death worldwide. Many therapeutic strategies have been developed to reduce maternal morbidity and mortality like oxytocin, prostaglandin, and uterine balloons. A new member of the therapeutic arsenal has recently emerged, the chitosan (Celox®), used since several years by military doctors to stop bleeding of combat wounds. In 2012, a first study was reported with the successful use of chitosan-coated gauze to treat severe postpartum hemorrhage. We report here three cases of the use of chitosan to treat life-threatening obstetric bleeding. In the first case, a pelvic packing with chitosan gauze after hemostatic hysterectomy with persistent bleeding. In the second case, the use of chitosan powder in a case of severe bleeding from multiple vaginal tears. In the third case, the use of chitosan gauze in uterine packing for postpartum hemorrhage by atonia. Postpartum hemorrhage of uterine origin resistant to treatment with prostaglandins can be treated with chitosan-coated gauze. This treatment requires no training and its costs are one fifth those of a Bakri® intrauterine balloon. Using these two forms of chitosan, powder and gauze, we have developed a new therapeutic method at our disposal for dealing with the most serious cases of bleeding.

KEYWORDS: Chitosan; Postpartum hemorrhage; Uterovaginal packing.

INTRODUCTION

Postpartum hemorrhage (PPH) is a major cause of maternal death worldwide, causing about 127,000 deaths per year.[1] The WHO defines PPH as the loss of more than 500 mL of blood for a vaginal delivery, and more than 1000 mL of blood after cesarean section, in the first 24 hours after delivery. Many therapeutic strategies have been developed to reduce maternal morbidity and mortality and to avoid the need for radical and invasive procedures, such as hysterectomy. Treatments include oxytocin, prostaglandin analogs, and ergot derivatives. In addition to pharmacological treatments, uterine packing techniques, such as Bakri® balloons, are used. A new member of the therapeutic arsenal has recently emerged – chitosan (Celox®) – used in two principal forms as a hemostatic agent: a powder and hemostatic gauze. Chitosan is a hydrophilic biopolymer that comes from chitin of crustacean shells. Its hemostatic mode of action is due to electrostatic interaction with red blood cell membranes.[2] This product has been used for several years by military doctors, to stop the bleeding of combat wounds. In 2012 and 2013, Schmid et al reported the first case of the successful use of chitosan-coated gauze to treat severe PPH.[3,4]

We report here three cases of the use of chitosan to treat life-threatening obstetric bleeding.

Clinical Case 1: Pelvic packing with chitosan hemostatic gauze after cesarean section.

Mrs. B. was 35 years old and in her 10th pregnancy. She already had seven live-born children, four of whom were delivered by cesarean section. The monitoring of her pregnancy was poor, with only one ultrasound scan, yielding normal results, at 36 weeks of gestation. This patient presented at the emergency room in early labor at 37 weeks + 4 days of gestation. Hemo-

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-129

stasis results were normal and hemoglobin concentration was 10.5 g/100 mL. An emergency cesarean section was performed, resulting in the delivery of a newborn weighing 2790 g newborn and the discovery of placenta accreta. The patient displayed extensive bleeding and hemodynamic instability, necessitating hysterectomy. Despite the transfusion of packed red blood cells and fresh frozen plasma, the patient continued to bleed from the edge of the cervix and a layer within the pelvic cavity. The bleeding was not stopped by hemostatic sutures, or by the compression of this pelvis displaying multiple adhesions with surgical sheets, and the patient's hemodynamic status worsened. We, therefore, decided to attempt the packing of the pelvic cavity with chitosan-coated gauze. The bleeding stopped completely, within five minutes. The gauze was left in place and the wall of the cavity was closed. Postoperative hemoglobin concentration was 4.5 g/100 mL, and the patient presented major hemostatic problems (Quick's test: 25%; platelet count 24000/mm³. She received six units of packed red blood cells and four of fresh frozen plasma. The gauze was removed, without difficulty, during a surgical intervention two days later. We observed no specific inflammatory response to contact with the gauze. The biological and clinical progression of the patient was satisfactory with normal coagulation tests after 24 hours, and she was released from hospital seven days after surgery, following an abdominopelvic CT scan revealing an absence of abnormalities.

Clinical Case 2: Use of chitosan powder in a case of severe bleeding from multiple vaginal tears.

Mrs D. was a young patient (18 years old) in her first pregnancy, with no particular medical or surgical antecedents. Her pregnancy had been monitored correctly and the various obstetric scans were all normal. She presented with preeclampsia in late pregnancy, for which labor was triggered with misoprostol. Hemoglobin concentration at admission was 11.3 g/100 mL. The patient was treated with a combination of urapidil and magnesium sulfate, due to a worsening of the clinical signs of preeclampsia during labor. During the second stage of labor, the fetus presented an abnormal heart rate, prompting vacuusm extraction through the pelvis. The newborn weighed 3220 g at birth, had Apgar score of 2/5/6, and a cord blood lactate concentration of 5.6 mmol/L. Delivery was aided by the intravenous injection of 5 IU of oxytocin during clearance of the shoulders. The placenta was considered to be complete on examination. The immediate postpartum period was normal, but the patient displayed heavy bleeding 12 hours later. We estimated that she had lost more than 2 liters of blood and her hemoglobin concentration fell to 5.7 g/100 mL. A uterine examination was performed under general anesthesia, leading to the removal of an abnormal cotyledon and numerous clots.

Examination of the birth canal revealed several vaginal tears that were bleeding heavily. Hemostatic sutures and manual compression were insufficient to stop the bleeding. Biological tests revealed a hemoglobin concentration of 4.2 g/100 mL and disturbed hemostasis. The patient presented hemodynamic instability with low blood pressure, requiring the transfusion of three units of packed red blood cells and two of fresh frozen plasma. Given the persistent bleeding, chitosan powder was applied to the hemorrhagic vaginal tears, which were then subjected to compression with gauze for five minutes. No further vaginal bleeding was observed after removal of the gauze and hemostatic tests was normal after 2 days. The patient was able to leave the hospital five days later, and vaginal examination showed good healing with no signs of an inflammatory reaction to chitosan. To our knowledge, this is the first case of the use of powdered chitosan on multiple vaginal lesions in context of PPH.

Clinical Case 3: Uterovaginal packing for PPH.

Mrs. P., 25 years of age, fourth pregnancy, with three previous live births and no particular medical or surgical antecedents, presented with spontaneous labor at 40 weeks + 2 days of gestation. Labor was normal, with the spontaneous delivery of a baby girl weighing 3115 g. The delivery was aided by the intravenous injection of 10 IU oxytocin. The patient then displayed abnormal bleeding, with an estimated blood loss of 800 mL. A uterine examination was carried out, including the birth canal and the cervix, and no abnormalities were found. The bleeding persisted and the patient received another injection of 10 IU oxytocin and a perfusion of one ampoule of sulprostone over the course of an hour. Despite this treatment, the patient continued to bleed heavily, due to uterine atonia. We decided to pack the uterus with chitosan-coated gauze. The bleeding stopped immediately. The patient had a hemoglobin concentration of 11.5 g/100 mL on admission, and of 8.6 g/100 mL after the bleeding was stopped. Her total blood loss was estimated at 1.2 L. The gauze was removed the day after delivery, by simply pulling it out of the uterine cavity. The postpartum period was otherwise unremarkable.

DISCUSSION

Chitosan is a hydrophilic biopolymer obtained by the chemical deacetylation of chitin, the principal component of crustacean shells. Its hemostatic mode of action is independent of the coagulation cascade. It effects are due to electrostatic interactions with red blood cell membranes.[2] Chitosan coagulates blood, even in the presence of heparin, and has antibacterial properties, reducing the risk of infection.[5,6] It is used by the British and American armies to achieve rapid and efficient hemostasis in the battlefield, mostly to control bleeding due to bullet wounds. Chitosan exists in three forms: granules, gauze, and nasal plugs.

Most studies evaluating the hemostatic activity of chitosan have been carried out on laboratory animals and have yielded spectacular results.[7,8] The few studies performed on humans essentially concerned military personnel in war zones.[9-11] The rapid hemostatic action of chitosan considerably reduces bleeding, facilitating the transport of the wounded to the operating theater. The largest series of traumatic injuries in civilians treated with chitosan was performed by Hatamabadi et al. It involved 160 patients and concluded that chitosan resulted in faster hemostatic control than a conventional compression bandage, with no adverse effects.[12] Other uses are being developed,

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-129

including the maintenance of hemostasis during surgery[13] or the prevention of recalcitrant epistaxis.[14]

Only one group has evaluated a hemostatic chitosan dressing for PPH. The group of Schmid et al[4] obtained good results for uterovaginal packing with chitosan in 18 of 19 cases. The rate of hysterectomy in this department has decreased by 75% in 18 months (OR 4.27; p=0.044).

PPH of uterine origin resistant to treatment with prostaglandins can be treated with chitosan-coated gauze. This treatment requires no training and its costs are one fifth those of a Bakri® intrauterine balloon. We report here three types of life-threatening obstetric hemorrhage for which chitosan rapidly stopped the bleeding. Using these two forms - powder and gauze, we have a new therapeutic arsenal at our disposal for dealing with the most serious cases of bleeding. Chitosan-coated gauze could also be used to treat extensive vulvar hematomas, which are often difficult to treat surgically.

CONCLUSION

We report three cases of severe obstetric hemorrhage resolved by the use of chitosan. Chitosan thus constitutes a new alternative for the treatment of all forms of severe bleeding. It is inexpensive, its use requires no training, and could be made available in developing countries. Larger comparative studies are required to determine the place of chitosan treatment among the resources already at our disposal.

CONFLICTS OF INTEREST: None.

CONSENT

The patients had provided permission for publication of their case details.

AUTHORS' CONTRIBUTION

G. Carles and C. Dabiri contributed equally to this work.

REFERENCES

1. World Health Organization. Reducing the global burden: Postpartum haemorrhage. 2008.

2. Zhao Y, Park R-D, Muzzarelli RAA. Chitin deacetylases: properties and applications. *Mar Drugs*. 2010; 8(1): 24-46. doi: 10.3390/md8010024

3. Schmid BC, Rezniczek GA, Rolf N, Maul H. Postpartum hemorrhage: use of hemostatic combat gauze. *Am J Obstet Gynecol*. 2012; 206(1): e12-e13. doi: 10.1016/j.ajog.2011.09.018

4. Schmid BC, Rezniczek GA, Rolf N, Saade G, Gebauer G, Maul H. Uterine packing with chitosan-covered gauze for control of postpartum hemorrhage. *Am J Obstet Gynecol*. 2013; 209(3): 225.e1-e5. doi: 10.1016/j.ajog.2013.05.055

5. Kozen BG, Kircher SJ, Henao J, Godinez FS, Johnson AS. An alternative hemostatic dressing: comparison of CELOX, HemCon, and QuikClot. *Acad Emerg Med*. 2008; 15(1): 74-81. doi: 10.1111/j.1553-2712.2007.00009.x

6. Tan H, Ma R, Lin C, Liu Z, Tang T. Quaternized chitosan as an antimicrobial agent: antimicrobial activity, mechanism of action and biomedical applications in orthopedics. *Int J Mol Sci*. 2013; 14(1): 1854-1869. doi: 10.3390/ijms14011854

7. Klokkevold PR, Subar P, Fukayama H, Bertolami CN. Effect of chitosan on lingual hemostasis in rabbits with platelet dysfunction induced by epoprostenol. *J Oral Maxillofac Surg*. 1992; 50(1): 41-45. doi: 10.1016/0278-2391(92)90194-5

8. Kunio NR, Riha GM, Watson KM, Differding JA, Schreiber MA, Waters JM. Chitosan-based advanced hemostatic dressing is associated with decreased blood loss in a swine uncontrolled hemorrhage model. *Am J Surg*. 2013; 205(5): 505-510. doi: 10.1016/j.amjsurg.2013.01.014

9. Alam HB, Burris D, DaCorta JA, Rhee P. Hemorrhage control in the battlefield: role of new hemostatic agents. *Mil Med*. 2005; 170(1): 63-69. doi: 10.7205/MILMED.170.1.63

10. Pozza M, Millner RWJ. Celox (chitosan) for haemostasis in massive traumatic bleeding: experience in Afghanistan. *Eur J Emerg Med*. 2011; 18(1): 31-33. doi: 10.1097/MEJ.0b013e32833a5ee4

11. Arul GS, Bowley DM, DiRusso S. The use of Celox gauze as an adjunct to pelvic packing in otherwise uncontrollable pelvic haemorrhage secondary to penetrating trauma. *J R Army Med Corps*. 2012; 158(4): 331-333. doi: 10.1136/jramc-158-04-12

12. Hatamabadi HR, Asayesh Zarchi F, Kariman H, Arhami Dolatabadi A, Tabatabaey A, Amini A. Celox-coated gauze for the treatment of civilian penetrating trauma: a randomized clinical trial. *Trauma Mon*. 2015; 20(1): e23862. doi: 10.5812/traumamon.23862

13. Huang X, Sun Y, Nie J, et al. Using absorbable chitosan hemostatic sponges as a promising surgical dressing. *Int J Biol Macromol*. 2015; 75: 322-329. doi: 10.1016/j.ijbiomac.2015.01.049

14. Kourelis K, Shikani AH. Effectiveness of chitosan-based packing in 35 patients with recalcitrant epistaxis in the context of coagulopathy. *Clin Otolaryngol*. 2012; 37(4): 309-313. doi: 10.1111/j.1749-4486.2012.02488.x

GYNECOLOGY AND OBSTETRICS RESEARCH

Openventio PUBLISHERS

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-130

Case Report

*Corresponding author
Solwayo Ngwenya, MBChB, DFSRH, MRCOG
Consultant Obstetrician and Gynaecologist
Head of Department of Obstetrics and Gynaecology
Mpilo Central Hospital, Vera Road;
Mzilikazi Founder and Chief Executive Officer, Royal Women's Clinic
52A Cecil Avenue, Hillside
Bulawayo, Matabeleland, Zimbabwe
E-mail: drsolngwe@yahoo.co.uk

Volume 3 : Issue 1
Article Ref. #: 1000GOROJ3130

Article History
Received: June 13th, 2016
Accepted: July 18th, 2016
Published: July 18th, 2016

Citation
Ngwenya S. Placental retention with accreta in a uterine anomaly. *Gynecol Obstet Res Open J*. 2016; 3(1): 16-18. doi: 10.17140/GOROJ-3-130

Placental Retention with Accreta in a Uterine Anomaly

Solwayo Ngwenya, MBChB, DFSRH, MRCOG*

Consultant Obstetrician and Gynaecologist, Head of Department of Obstetrics and Gynaecology, Mpilo Central Hospital, Vera Road; Founder and Chief Executive Officer Royal Women's Clinic, 52A Cecil Avenue, Hillside, Bulawayo, Matabeleland, Zimbabwe

ABSTRACT

A 32 year-old P_1G_2 went into spontaneous labour at 37 weeks gestation. Precipitate labour progressed to a normal vaginal delivery (NVD) of a baby boy within 2 hours of onset. She had a retained placenta. The patient was taken to theatre for an examination under anaesthesia and manual removal of placenta. It was discovered that she had uterine anomaly. A calcified accretic placenta was manually removed. The patient was kept on an oxytocin infusion overnight and given intravenous broad spectrum antibiotics. She made a good recovery and was discharged home after 3 days on oral antibiotics.

KEYWORDS: Retained placenta; Uterine anomalies; Life-threatening; Catastrophic haemorrhage; Reproductive outcomes; Maternal mortality.

INTRODUCTION

A retained placenta is a life-threatening condition as it can cause catastrophic haemorrhage and maternal mortality and morbidity. It complicates 2-3% of vaginal deliveries and causes postpartum haemorrhage. Its management must be prompt and effective. The prevalence of uterine anomalies in the general population is 1:201 (0.50%), the commonest types being septate (34%) and bicornuate (39%) of all cases of uterine anomalies.[1] Congenital uterine anomalies are associated with the highest incidence of reproductive failure and obstetric complications.[2] There is scanty data on uterine anomalies and retained placenta in the literature. A retained placenta in a rudimentary horn of a double uterus was described by Wilson in 1955.[3] It is therefore important to document more cases as to alert clinicians and help save lives.

CASE REPORT

A 32 year-old P_1G_2 went into spontaneous labour at 37 weeks gestation. She had had a normal antenatal period. In her previous obstetric history, she had delivered NVD 8 years ago a baby boy birth weight 3000 g. The couple had struggled to conceive but had not consulted a clinician to undergone any investigations for subfertility. She had no other medical or surgical history.

Precipitate labour progressed to a NVD of a baby boy within 4 hours of onset. The baby's birth weight was 2710 g. The Apgar scores were 8, 9 and 10 at 1, 5 and 10 minutes respectively. The third stage of labor was managed actively by giving oxytocin 10 international unit (IU) at the delivery of the anterior shoulder. Gentle cord traction was attempted but there were no signs of placental separation after 15 minutes. There was minimum bleeding per vagina. An oxytocin infusion of 40 IU in a litre of normal saline was commenced. After 30 minutes post-delivery, there were still no signs of placental separation. Another attempt at gentle cord traction led to the cord snapping.

The patient was taken to theatre for an examination under anaesthesia and manual removal of placenta. There were second degree tears in the vulva and the cervical cervix or cervical os had contracted significantly. On insertion of the hand, it went right into the fundus

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-130

and no placenta was felt. The empty cavity was roomy. While moving the hand inside a second opening was felt (Figure 1). This was a case of uterine anomaly only detected in theatre. The newly discovered uterine cavity went right up to the costal margin. There at the fundus an accretic placenta was found. The placenta was sheared off and manually removed. It was a calcified placenta. The second degree tears were repaired. The patient was kept on an oxytocin infusion overnight and given intravenous broad spectrum antibiotics. She made a good recovery and was discharged home after 3 days on oral antibiotics.

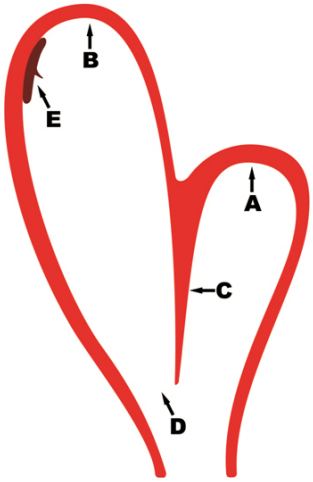

Figure 1: Showing the clinical impression of what was discovered in theatre during examination under anaesthesia. The hand first went into the fundus of the left uterine horn (A), there was a septum (C) another opening (D) was discovered leading the fundus of the right uterine horn (B). The placenta was found high up (E), accretic in the right uterine horn.

DISCUSSION

Retained placenta poses great danger to maternal health as it can lead to catastrophic haemorrhage, the risk is even higher with an adherent placenta. Prenatal diagnosis of abnormal placentation allows anticipation of multidisciplinary team[4] management that prevents adverse outcomes. It is important that it is recognized early and prompt steps taken to have it manually removed under anaesthesia. An association of a retained placenta and uterine anomaly has not been described in the literature hence this case is to highlight this association. Uterine morphology can be ascertained outside pregnancy by hysterosalpingography and laparoscopy.[5] Magnetic resonance imaging (MRI) can also be a useful tool to diagnosis. Some uterine anomalies may permit normal obstetric outcomes.[5]

Women with congenital uterine malformations usually have higher incidence of subfertility and complications during pregnancy and delivery.[6] The risks include preterm pre-labour rupture of membranes, small for gestational age babies and preterm delivery.[7,8] There are also risks of malpresentation and caesarean section (C-section) delivery,[7] and rupture of rudimentary uterine horn.[9] The complication of a retained placenta in uterine horn is not described in the literature. Uterine anomalies are associated with both normal and adverse reproductive outcomes.[10]

CONCLUSION

This case reminds clinicians of rare clinical associations that lurk underneath the surface undetected and yet pose significant danger to maternal health. When faced with unusual clinical findings, clinicians must explore other areas that may lead to the discovery of unexpected pathology. This can be life-saving.

REFERENCES

1. Nahum GG. Uterine anomalies: How common are they, and what is their distribution among subtypes? *J Reprod Med*. 1998; 43(10): 877-887. Web site. http://europepmc.org/abstract/med/9800671. Accessed June 12, 2016

2. Zhang Y, Zhao YY, Qiao J. Obstetric outcome of women with uterine anomalies in China. *Chin Med J (Engl)*. 2010; 123(4): 418-422. Web site. http://124.205.33.103:81/ch/reader/view_abstract.aspx?file_no=201022059246370&flag=1. Accessed June 12, 2016

3. Wilson TM. Retained placenta incarcerated in the rudimentary horn of a double uterus. *Am J Obstet Gynecol*. 1955; 70(3): 669-671. Web site. http://www.ncbi.nlm.nih.gov/pubmed/13238517. Accessed June 12, 2016

4. Rao KP, Belogolovkin V, Yankowitz J, Spinnato JA. Abnormal placentation: Evidence-based diagnosis and management of placenta previa, placenta accrete, and vasa previa. *Obstet Gynecol Surv*. 2012; 67(8): 503-519. doi: 10. 1097/OGX.Ob013e3182685870

5. Raga F, Bauset C, Remohi J, Bonilla-Musoles F, Simon C, Pellicer A. Reproductive impact of congenital mullerian anomalies. *Hum Reprod*. 1997; 12(10): 2277-2281. doi: 10.1093/humrep/12.10.2277

6. Ma S, Bian X, Lang J. Pregnancy and its outcome in women with malformed uterus. *Zhonghua Yi Xue Za Zhi*. 2001; 81(7): 415-417. Web site. http://europepmc.org/abstract/med/11798909. Accessed June 12, 2016

7. Hiersch L, Yeoshoua E, Miremberg H, et al. The association between mullerian anomalies and short-term pregnancy outcome. *J Matern Fetal Neonatal Med*. 2016; 29(16): 2573-2578. doi: 10.3109/14767058.2015.1098613

8. Hua M, Odibo AO, Longman RE, Macones GA, Roehl KA, Cahill AG. Congenital uterine anomalies and adverse pregnancy outcomes. *Am J Obstet Gynecol*. 2011; 205(6): 558.e1-558.e5. doi: 10.1016/j.ajog.2011.07.022

9. Pal K, Majumdar S, Mukhopadhyay S. Rupture of rudimentary uterine horn pregnancy at 37 weeks geststion with fetal survival. *Arch Gynecol Obstet*. 2006; 274(5): 325-326. doi: 10.1007/s00404-006-0170-y

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-130

10. Rackow BW, Arici A. Reproductive performance of women with mullerian anomalies. *Curr Opin Obstet Gynecol*. 2007; 19(3): 229-237. doi: 10.1097/GCO.0b013e32814b0649

GYNECOLOGY AND OBSTETRICS RESEARCH
Openventio PUBLISHERS
ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-131

Mini Review

*Corresponding author
Parveen Parasar, BVSc, MVSc, PhD
Boston Center for Endometriosis
Brigham and Women's Hospital
Boston Children's Hospital
Harvard Medical School
Boston, MA 02115, USA
E-mail: suprovet@gmail.com

Volume 3 : Issue 1
Article Ref. #: 1000GOROJ3131

Article History
Received: June 23rd, 2016
Accepted: July 27th, 2016
Published: July 28th, 2016

Citation
Parasar P. High-throughput next generation sequencing: Applications in reproductive diagnosis and research. *Gynecol Obstet Res Open J*. 2016; 3(1): 19-22. doi: 10.17140/GOROJ-3-131

High-Throughput Next Generation Sequencing: Applications in Reproductive Diagnosis and Research

Parveen Parasar, BVSc, MVSc, PhD*

Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA

INTRODUCTION

The genetic information contained within a cell is transferred through the process of transcription of genes within a genome to produce messenger RNAs (mRNAs) and translation of mRNAs to synthesize proteins. The central dogma pathway represents all three stages of replication, transcription, and translation in the pathway:

DNA → RNA → Protein

Genes in a genome can be identified by creating a complementary DNA (cDNA) library from the pool of ribonucleic acid (RNA) transcripts. To generate a cDNA library, the RNA transcripts from a tissue or from cells are copied into more stable cDNA molecules, which are then stored into an appropriate vector to generate a collection of cDNA clones. The single pass, short 300-500 nucleotide sequences obtained from sequencing either end of the cDNA insert are called expressed sequence tags (ESTs). ESTs can be generated from the cDNA libraries obtained from the patient tissue/samples. Those ESTs can be used to determine the genes that express them and to determine whether they possess any nucleotide or single nucleotide polymorphisms (SNPs) in comparison to normal individual. The sites where deoxyribonucleic acid (DNA) sequences are different at a single nucleotide are called SNPs. Similarly, capability to detect and identify mutations in genes has been utilized by high-throughput sequencing methods.

Predominantly, Sanger method of sequencing is used for DNA sequencing and is the method routinely used for the past 38 years. Sequencing methodologies have been rapidly improved creating very powerful tools for detection and identification of SNPs and various infectious agents. Sanger sequencing process is carried out in a liquid phase where a predefined gene can be targeted in one run allowing extremely low rates of false positive and negative errors and producing high specificity. However, the process is very time consuming, especially when multiple genes are to be studied in a sample to detect variants underlying a disease.

Attempts to sequence larger genomes such as the whole genomes of various animal species, using multicapillary sequencing faced considerable caveats in scalability, speed and resolution. Subsequent major advances in technologies such as cyclic-array sequencing gave rise to 'second-generation or next-generation sequencing (NGS)'. In these technologies, repeated cycles occur during which DNA sequences, immobilized on a solid substrate, and are determined one base position at a time with the use of enzymatic manipulation and imaging-based data acquisition.

NEXT GENERATION SEQUENCING AS A DIAGNOSTIC TOOL

Second generation sequencing also called as NGS sequencing was first reported in 2005. It allows for great increase in throughput and potential cost reduction. With the introduction of next generation sequencing it is now possible to concomitantly sequence multiple genomes in less than two weeks all in single run. There are three overall processes that have been developed in NGS: whole genome sequencing (WGS), whole exome sequencing (WES) and targeted gene

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-131

sequencing (TGS).[1] WGS is capable of sequencing the entire genome in a single run while WES and TGS are more focused. WES focuses on only the protein coding regions, and TGS is the most focused and examines specific genes or regions of interest making it of key interest to clinical investigations (Figure 1). These advancements in biotechnology have been applied to the study of genetic diseases in animals and thus revolutionized the study of biological and evolutionary processes at the molecular level.

Contrary to earlier techniques, NGS reports all nucleotide sequences present in the original sample by enabling deeper sequencing at a faster and economically affordable rate thus improving the likelihood of identifying novel mutations and genetic variations. Furthermore, sampling, sample preparation and enrichment protocols have significant effects on the outcome of NGS-based diagnostics (Figure 2).

Sample Preparation

Sample preparation is composed of individual steps *viz.* homogenization, filtration, and nuclease treatment including nucleic acid extraction and purification followed by amplification. Homogenates and fluids must be centrifuged and microfiltered to get rid of free and released fine particles from larger particles. Enrichment is performed with the use of nuclease treatment. Deoxyribonuclease (DNase) and ribonuclease (RNase) are used in combination or alone to eliminate host contaminants.

Library Construction and Sequencing

Genomic sequences in samples are converted into sequencing libraries apt for cluster generation and sequencing. This process involves following steps:

a. fragmentation of DNA (mechanical or enzymatic shearing).
b. end-repair, modification and ligation of adapters to amplify sheared DNA by adapter- specific primers.
c. size-selection of DNA molecules with an optimal length for the current application of instrument, and
d. enrichment of adapter-ligated DNA by PCR (if the amount of source material is limited).

The constructed libraries are immobilized on a solid

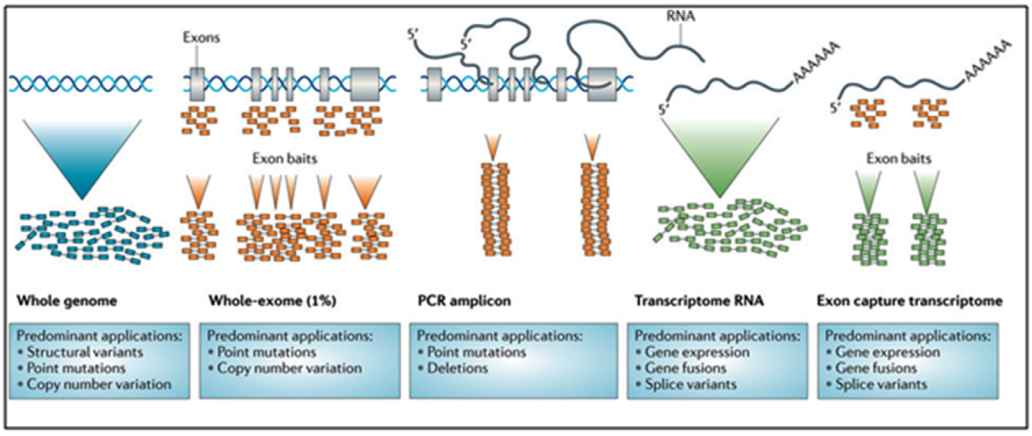

Figure 1: Applications of next-generation sequencing (NGS).[2]

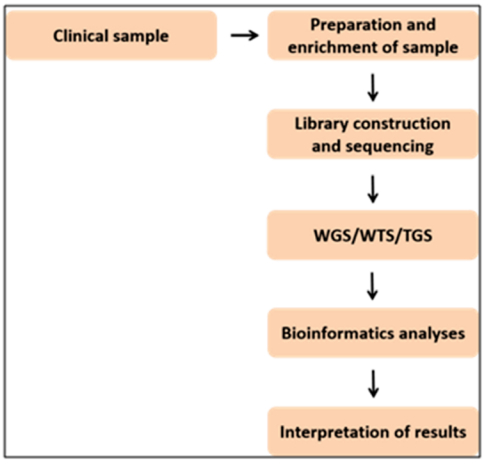

Figure 2: Next-generation sequencing workflow.

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-131

substrate for clonal amplification to generate distinct clusters of DNA copies. The commonly used solid platform are beads inside aqueous reaction bubbles (emulsion PCR or emPCR such as in 454 Pyrosequencing machine from Roche), glass flow cell (solid such as in Illumina machine from Illumina Inc.), ion-based sequencing chips (such as Ion Torrent from Life Technologies) etc.

Bioinformatics

The most common type of the data output from the machine is in the Fast Q format. Post-acquisition of data, researcher performs a quality control on the dataset to establish that the experiment or sample was processed according to standard parameters. Subsequently, data are screened for irrelevant sequence information, such as non-specific contaminants, and tag sequences. DNA or RNA sequence data are mapped towards the host genome or the known homologue. In the absence of homologue, data are processed through an algorithm to form larger sets of continuous reads or contigs. Contigs are them mapped towards the closest relative homologue to form a draft genome.[3,4] Bioinformatics tools enable targeted sequencing of RNA (RNA-seq) which not only maps the available mRNA sequence but also non-coding RNAs such as micro RNA (miRNA), small interfering RNA (siRNA, long non-coding RNA (lncRNA), and ribosomal RNA (rRNA). Consequently, next generation sequencing and associated bioinformatics is an efficient tool for visualizing RNA content within a sample.

APPLICATIONS OF NEXT GENERATION SEQUENCING IN OBSTETRICS AND GYNECOLOGY RESEARCH

Next-generation sequencing has been pivotal in diagnosis of known and emerging new mutations and SNPs that correlate with the susceptibility to disease occurrence. This technology enables researchers to identify gene-polymorphisms. NGS has been employed in many areas in medicine including cancer research, personalized-precision medicine and reproductive medicine and obstetrics and gynecology research.[5] Below are some examples of applications of NGS in reproductive diagnostics and gynecologic research.

Preimplantation Genetic Screening (Pgs)/Pre-Implantation Genetic Diagnosis (PGD)

Preimplantation genetic diagnosis eliminates the risk of passing genetic disorders to new born even prior to the woman becoming pregnant. This can be accomplished by examination and identification of potential genetic defects in developing embryo. With the increased age the aneuploidy of embryos increases which results in miscarriage, congenital abnormalities an implantation failure. Patients of recurrent implantation failure and recurrent miscarriages would benefit by Preimplantation genetic screening as it will improve clinical outcomes by increasing implantation rates and reducing miscarriage rates. Million of DNA fragments from embryos can be analyzed using NGS with greater sensitivity.[6-8]

Non-Invasive Prenatal Testing (NIPT)

Cell-free DNA (cfDNA) is now-a-days used as an advanced screening tool to determine fetal aneuploidy. Amount of fetal DNA from each chromosome is proportional to that of the mother in a normal pregnancy which is deviated slightly in an aneuploid pregnancy. For example, in Down syndrome, chromosome 21 has 3 copies of fetal DNA instead of normal 2 fetal copies. NGS allows detection and identification of such minute variations by sequencing and enumerating millions of cfDNA fragements from maternal plasma.[9,10]

Prenatal Diagnosis of Rare Genetic Diseases

NGS is now commonly used to sequence the protein coding genes collectively known as exome (whole exome sequencing (WES)) and is very powerful tool to detect many unknown and new genetic disease.[11]

Endometrial Receptivity Analysis (ERA)

Endometrial receptivity and implantation are critical features for a successful pregnancy outcome. ER is a characteristic phenotype which allows impantation and adhesion of embryo with the endometrium. With the advancements in transcriptomic analyses the genetic signatures of viable and potentially successful phenotype of endometrium can be predicted which leads to improved assisted reproductive technologies (ART) with better outcomes.[12] NGS-transcriptomics allows characterization of genes even at the miRNA level to establish sample-specific molecular profile in embryonic cells. This profile could be used as biomarkers defining a successful biological process or a disease. Consequently, this concept is useful in determining a window for successful transfer of embryo in ART and *in vitro* fertilization (IVF) techniques.[13]

One study reported the immensely usefulness of this high-throughput technology in targeting genes and molecular markers in obstetrics gynecological cancers such as ovarian cancer. NGS has been applied to analyze TGFβ/SMAD4 targets in ovarian cancer to identify genome-wide SMAD4 targets in epithelial ovarian cancer and aberrant TGFβ/SMAD4 signaling in ovarian tumorigenesis.[6] Taken together, NGS is robust technique and has great potential for reproductive diagnostics to detect genetic diseases. In addition, it is robust tool to control and monitor the genetic diversity of viral, bacterial and other causative agents that infect reproductive system and identification of potentially genetic variants.

These advantages as screening and characterization tool make next-generation sequencing ideal for vaccine development and quality control. Collectively, these merits demonstrate the new possibilities opened up by the NGS and metagenomics analyses to study known as well as new emerging diseases in domestic and wild animal populations.

The combination of diverse molecular biology and ge-

ISSN 2377-1542 Open Journal http://dx.doi.org/10.17140/GOROJ-3-131

nomics skills in multidisciplinary fashion is very important to enhance and extend our ability to develop effective and accurate diagnostic tools and disease control measures.

REFERENCES

1. Cordero P, Ashley EA. Whole-genome sequencing in personalized therapeutics. *Clin Pharmacol Ther*. 2012; 91(6):1001-1009.

2. Simon R, Roychowdhury S. Implementing personalized cancer genomics in clinical trials. *Nat Rev Drug Discov*. 2013; 12(5): 358-369. doi: 10.1038/nrd3979

3. Yegnasubramaniam S. Explanatory chapter: Next generation sequencing. *Methods Enzymol*. 2013; 529:201-208. doi: 10.1016/B978-0-12-418687-3.00016-1

4. Rehm HL, Bale SJ, Bayrak-Toydemir P, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*. 2013; 15(9): 733-747. doi: 10.1038/gim.2013.92

5. Bombard Y, Bach PB, Offit K. Translating genomics in cancer care. *J Natl Compr Cancer Netw.* 2013; 11: 1343-1353.

6. Kennedy BA, Deatherage DE, Gu F, et al. ChIP-seq defined genome-wide map of TGFβ/SMAD4 targets: Implications with clinical outcome of ovarian cancer. *PLoS One.* 2011; 6(7): e22606. doi: 10.1371/journal.pone.0022606

7. Hallam S, Nelson H, Greger V, et al. Validation for clinical use of, and initial clinical experience with, a novel approach to population-based carrier screening using high-throughput, next-generation DNA sequencing. *J Mol Diagn.* 2014, 16: 180-189. doi: 10.1016/j.jmoldx.2013.10.006

8. Fiorentino F, Bono S, Biricik A, et al. Application of next-generation sequencing technology for comprehensive aneuploidy screening of blastocysts in clinical preimplantation genetic screening cycles. *Hum Reprod*. 2014; 29: 2802-2813. doi: 10.1093/humrep/deu277

9. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. *New Engl J Med.* 2014; 370(9). Web site. http://www.nejm.org/doi/pdf/10.1056/nejmoa1311037. Accessed June 2nd, 2016

10. Norton ME, Jacobsson B, Swamy GK, et al. Cell-free DNA analysis for noninvasive examination of trisomy. *New Engl J Med.* 2015; 372: 1589-1597. doi: 10.1056/NEJMoa1407349

11. Farwell KD, Shahmirzadi L, El-Khechen D, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model–based analysis: Results from 500 unselected families. *Genet Med.* 2014; 17(7): 578-586. doi: 10.1038/gim.2014.154

12. Díaz-Gimeno P, Ruíz-Alonso M, Blesa D, Simón C. Transcriptomics of the human endometrium. *Int J Dev Biol*. 2014; 58(2-4): 127-137. doi: 10.1387/ijdb.130340pd

13. Werner T. Next generation sequencing allows deeper analysis and understanding of genomes and transcriptomes including aspects to fertility. *Reprod Fertil Dev*. 2011; 23(1): 75-80. doi: 10.1071/RD10247