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# ART PROCEDURE AND GENOMIC IMPRINTING: ANY CONCERN FOR THE SUB-SAHARAN AFRICA SETTING

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**ABSTRACT:** *ART has become the mainstay treatment modality for significant number of infertile couples.it involves numerous steps culminating into subjecting the gametes and early developing embryos to some forms of environmental stress. ART procedures tend to coincide with the period of DNA demethylation and methylation that form the basis of parental imprint during gametogenesis, fertilization and early embryonic development. Hence possible source of imprint disorder necessitating the need for investigating the association. Recent findings have demonstrated the association of ART and imprinting disorders ion animal studies. While case series from the registries suggest association in children with Beckwith-Wiedemann syndrome, Angelman syndrome and Retinoblastoma. Though, more recent studies could not substantiate it independent of other factors such as inherent infertility.*

**KEYWORDS:** ART, Epigenetic, Genomic Imprinting, Imprinting Disorder

## INTRODUCTION

Assisted reproduction technology (ART) has become widely used in the treatment of couples experiencing infertility problems. It involves the in-vitro manipulation of human gametes and preimplantation embryos at the time of regulating genetic information. (De Waal *et al*., 2015). Over the last decade, the practice of ART has rapidly increased in the sub-Saharan Africa. Though these techniques are often considered to be safe, a large number of reports have suggested that increased risk of adverse perinatal outcome and congenital anomalies through epigenetic disruption, are associated with their use(Ceelen *et al*., 2008). Epigenetics is a concept which gives the framework for how a variety of cell phenotypes develop from a single identical sequence of genetic code. It is the heritable changes in gene expression without a change in DNA sequence (Kohda *et al*., 2013). One of the characteristics of epigenetic is its gene expression regulation mechanism transmitted to the next generation through a phenomenon of genomic imprinting (Chiba *et al*., 2013).

### Ovarian Hyperstimulation

ART involve various procedures such as IVF and ICSI, which encompasses several steps of manipulation of the gametes and embryo. To date, it is not certain which ART procedures attribute to epigenetic disruption (Fauque, 2013). However, one of the important issues regarding possible epigenetic alterations is the artificial induction of ovulation. It has showed that human oocyte-like in a mouse model, are associated with an aberrant gain in methylation at H19 and loss of methylation at PEG1 after ovarian stimulation (Sato *et al*., 2007).These findings were froth with confounding factors such as the age of the patient and other factors inherent to female infertility. Also, Geuns et al. 2007 noted more methylated KCNQ10T1 DMR



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(KvDnMR1) in GV and metaphase 1 (M1) oocytes of the natural cycle than in the stimulated cycles suggestive of stimulation may disrupt dynamism of de novo methylation during oocyte maturation and consequently lead to recruiting of immature follicles. Similarly, analysis of 2 of 4 MII oocyte following ovarian stimulation revealed abnormally low methylation pattern for KCNQ10T1 (Fauque *et al*., 2013). While the molecular basis for this epigenetic disruption remains unclear, some believe may be due to developmental delay in the oocyte preventing imprint establishment at the right time (Ludwig 2005, Sutcliffe *et al*., 2006, Hajj *et al*., 2013).Thus, production of low-quality oocytes to maturity. Also, DNA methylation marks are laid in the both gametogenesis at different times. While that of the spermatogenesis occurred during the prenatal stage and completed postnatally, the oogenesis commences after puberty in the growing oocyte through primordial to antral follicles. Ovarian stimulation, therefore, may disrupt the process. Thus hinder the acquisition of imprint and consequently development of imprinting disorder (Fauque *et al*., 2013).

Furthermore, a study with mice revealed a high risk of fetal growth retardation with ovarian stimulation(Fortier *et al*., 2008). The epimutation noted here is associated with abnormal bi-allele expression of maternal and paternal imprinted genes (Snrpn and H19 genes) and as well as increased expression of the Igf2 gene in the placental tissue. Thus, superovulation may alter the maintenance of imprinting during preimplantation period, particularly in trophectoderm-derived tissues.The finding in this mouse model may have similar human clinical effect in ART concerning implantation failure and intrauterine growth restriction due to placental dysfunction (Rancourt *et al*., 2012).

**In-vitro maturation (IVM) and Culture media**

In-vitro growth (IVG) and in-vitro maturation (IVM) of oocytes haven been retrieved in the primordial or Germinal vesicle (GV) stages respectively and then cultured to complete the final steps of maturation may be associated with epigenetic disruption and interfere with normal imprint acquisition (Chiba et al. 2013). For example, a study on IVG with mouse oocyte has revealed aberrant methylation with the loss of methylation (Igf2 and Peg1/Mest ) and gain of methylation (H19) (Bonakdar *et al*., 2015). Similarly, demethylation at the Igf2r and Mest loci and gain in methylation at the H19 DMR has been shown in the extended culture of mouse oocyte which conflicted with the short culture condition (Hajj and Haaf 2013). Suggesting that the epigenetic defect noted may be due to the adverse effect of the culture environment often associated with prolonged culture (Ankaert *et al*., 2009).

The impact of culture effects on imprinted gene expression and epigenetic regulation revealed some indications suggestive of an association of epigenetic effects with specific types or formulations of culture media (Young and Beaujean 2004). An example is the impact of Whitten's medium for the culture of mouse embryo from 2 cell stage to Blastocyst with resultant aberrant H19 expression with loss of methylation in the H19 DMR (Laprise 2009) and activation of the silent paternal allele. There was, however, no effect on the imprinted gene Snrpn and in the activity of DNA methyltransferase 1(Fauque 2013). Also Dnmt3a methyltransferase, the Igf2r gene were upregulated in bovine embryos cultured in Charles Rosenkrans 1 (CR1aa) media as well as Potassium simplex optimized medium (KSOM) with amino acid content (Huntriss and Picton 2008). A genome-wide study two-cell mouse embryos have revealed that suboptimal culture media and/or presence of a toxic compound in the media can perturb the methylation reprogramming with subsequent developmental arrest and embryo loss (Zaitseva *et al*., 2007). Also, it has been shown that culture media can influence the parent-specific activity of the imprinted H19. Locus (Hajj and Haaf, 2013) and the change in paternally


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expressed Igf2 gene suggesting the influence of in vitro culture of mouse preimplantation embryo bias towards the aberrant expression of maternal allele (Rivera *et al*., 2008).

One striking example of the effect of culture on embryo development is the in vitro produced Lambs and calves with the exhibition of overgrowth abnormalities termed Large offspring syndrome (LOS) associated with in vitro culture induced overexpression and altered methylation of IGF2R(Farin *et al*., 2006). This large offspring is reminiscent of BWS in humans linked to IVM conditions (Hiendleder et al. 2006).Moreover, extended culture particularly media containing FCS (Niemann *et al*., 2010). It is interesting to note that IGF2R not imprinted in humans (Hori et al. 2010) and thus less susceptible to epigenetic misprogramming. Thus, explain the low absolute risk for BWS in ART children compared to LOS problem in Ruminants (Hori *et al*., 2010). Also, it has been shown that various culture media can alter the epigenetic mechanism that regulates imprinted gene expression, and this effect may be worse in the placenta (Mann et al. 2004). In this study, bi-allele expression and aberrant methylation of genes H19, Ascl2, Snrpn, Peg3, and Xist was noted in the placental tissue following cultured in Whitten's medium. Suggestive of a link between the aberration of imprinted gene expression and intrauterine growth restriction and low birth weight resulting from placental insufficiency (Laprise, 2009).

**Intracytoplasmic sperm injection(ICSI)**

Men with a suboptimal sperm count or quality are often offered intracytoplasmic sperm injection (ICSI) to enhance their potential for being able to father a child. ICSI has linked to AS (Kobayashi et al. 2007). However, sperm from oligospermic men displays imprinting defect in H19 and MEST (Laprise, 2009). Suggesting the possibility that the use of suboptimal sperm and not the ICSI may be the cause of AS. This procedure sometimes necessitates the use of immature sperm cells such as the Round spermatid injection (ROSI) as well as round spermatid nucleus injection (ROSNI), or secondary spermatocyte injection (SECSI) technique. The concern borders on the fact immature gametes may not have acquired all the epigenetic information required for normal development as evidenced by the findings that spermatogenesis -specific genes undergo late epigenetic reprogramming during spermiogenesis in the epididymis (Hartmann *et al.*, 2006). Furthermore, the spermatid genome is transcriptionally active, and its introduction into the oocyte may interfere with the epigenetic reprogramming during the pre-implantation stage resulting in an alteration in gene expression pattern of several genes in early embryo derived from ROSI (Robinson *et al*., 2005). Also, DNA methylation and histone methylation in zygotes from ROSI has shown a significant difference when compared to ICSI suggesting the insufficiency of the ROSI technique (Laprise 2009) with associated higher rate of developmental arrest of the embryo (Robinson *et al*., 2005).

**Ooplasmic Transfer**

In a bid to enhance embryo viability, the Ooplasmic transfer was first reported by Cohen and colleagues as a means of obtaining pregnancy in women with recurrent implantation failure (Yanagimachi 2005). The early debate concerning its safety centered on the possible effect of mitochondrial heteroplasmy (Hawes et al. 2002). Studies with a mouse have revealed that genetically diverse ooplasms can impose altered epigenetic modification on parental genomes resulting in a defect in gene expression and development (Yanagimachi 2005, Fauque 2013, De Waal, 2015) that can be heritable and observed in the next generation. Given this likely setback, more research needs to be done with a suitable animal model to establish the safety



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and efficacy of the method. Similarly, cloning is widely used for the multiplication genetically identical cells. Available data has shown that reprogramming is incomplete in most nuclear-transferred embryos resulting in disturbances in methylation dynamics (Romundstad *et al*., 2008). Evaluation of imprinted gene expression in cloned mice and its donor embryo stem cells (Esc) has showed variation in the expression of H19 and Peg1 genes between Es cells subclones (Roundstad *et al*., 2008) suggesting stem cells association with the unstable epigenetic state.

**Beckwith- Wiedemann syndrome (BWS)**

Beckwith-Wiedemann Syndrome (BWS) is one of the effects with current evidence associated with ART procedure and micromanipulation in humans characterized by overgrowth syndrome by macroglossia, macrosomia, and neonatal hypoglycaemia. The condition results from disruption of an imprinted gene on chromosome 11p15 with hypomethylation of the KvDMR1 imprinting region as the primary source of epimutation. Study has shown an incidence of 4% in children born after ART compared to approximately 1% expected in the general population (Maher *et al*., 2003). While a prevalence of 4.6% compared to a background rate of 0.8% as noted in another study (DeBaun *et al*., 2003). Also, Sutcliffe *et al*., 2006 reported a statistically significant increased occurrence of BWS amongst children born after ART procedure. Despite these findings, it was difficult to attribute the exact effect of the various protocol. In light of this, Chang et al., 2005 reported the 19 children from a BWS registry following ART had ovarian stimulation as the common parameters. However, given the small size of the study, a larger cohort of cases is needed to substantiate this. Furthermore, the significant findings of epigenetic defects in other DMR at IGF2R, SNRPN, and PEG1/MEST which observed in the natural conceived BWS patients give credence to more generalized global epigenetic defects in BWS. Thus, suggesting the epigenetic errors are likely due to failure to maintain maternal imprint after fertilization (Huntriss and Picton, 2008) and the mechanism attributed to inappropriate or defect in the DNMT1o methyltransferase during pre-implantation development (Eroglu and Layman, 2012). The fact that similar event occurs in the natural conceived BWS, ART procedure may have aggravated it.

**Angelman syndrome**

Some studies have reported a link between Angelman syndrome (AS) and ICSI (Rossignol *et al*., 2006, Manipalviran *et al*., 2009, Bonakdar *et al*., 2015). The first publication noted two children with significant hypomethylation at SNRPN imprinting control region on chromosome 15q11-15 (Manpalviran *et al*., 2009) which is a rare cause of AS. However, other reports did not establish evidence of a link between methylation defect at SNRPN after ICSI (Huntriss and Picton, 2008). A nation-wide survey in Netherland reported that 12 of 63 children with AS were born in families with fertility problems and had ovulation induction (Fauque, 2013).On the other hand, one study revealed the same risk for AS in children of infertile couples who had spontaneously achieved pregnancy (Ludwig *et al*., 2005). Suggesting that increased AS risk may be due to infertility problems in the couples. To date, there has been reported cases of AS following IVF or ICSI, and some of these children appeared to have an imprinting defect. Though, the number of cases in these studies were small due the rarity of the disease. The proportion of children with an imprinting defect as a cause of AS is higher than in the general population establish a link between ART and AS. Whether IVF, ICSI, or ovulation induction is the cause remain uncertain and further study is needed to unravel.



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**Silver-Russell syndrome (SRS)**

A recently reported case of Silver-Russell Syndrome (SRS) revealed hypermethylation at the PEG1/MEST DMR (Ceelen *et al*., 2008) suggesting that an aspect of ART treatment may aggravate this aberration at the paternal DMR. Abu-Amero et al. 2008 reported a case of SRS following ICSI. While other studies reported a total of 9 cases of SRS patients conceived with ART (Wakeling *et al*., 2010, Eroglu and Layman, 2012). Six of the 9 cases had an imprinting defect in DMR1 on chromosome 11p15 (Wakeling *et al*., 2010). However, there is no consensus on the evidence for or against an association between ART and imprinting defect in SRS due to the few number of cases.

Another evidence for the epigenetic effect of micromanipulation is a reported case of an undiagnosed overgrowth syndrome characterized by refractory seizures and developmental delay in a child conceived by ICSI (Shah *et al*., 2006). Others are oculo-auricular-vertebral spectrum (OAVS)/Goldenher syndrome (Huntriss and Picton, 2008), and Retinoblastoma reported in 8 children born through IVF suggestive epigenetic disruption from ART (Eroglu and layman, 2012).

Over the last two decades there have been a paradigm shift to ART in the management of infertile couples which accounts for 20-30 % in Nigeria (Nathan and Chikondi, 2016 ). Despite the recourse to this mode of treatment, there little or no documentation to its genomic implication in sub Saharan Africa. It therefore becomes imperative to create an awareness of ART in the genetic makeup.

## CONCLUSION

Considering the coincidence in the timing of ART and major epigenetic reprogramming events during gametogenesis/embryonic development, sustaining the epigenetic safety has become a major concern. It is possible that suboptimal condition in ART may induce disruption in the epigenetic process leading to abnormal development and imprinting disorders. Due to the variability in the ART protocols and the rarity of imprinting disorders, it is hard to determine the causative relationship between an increased risk for epigenetic disorders and ART procedures reliably. Furthermore, it is not certain whether the epimutation found in ART infants are as a result of ART procedures or are inherent in the infertility problems in the couple. Also, extrapolation of data from animal models may be inadequate due to significant discordance of imprinting status between imprinted genes and as well as variation in the regulation of epigenetic information in humans and other mammals (Laprise, 2009). However, despite some conflicting results, both human and animal studies suggest a possible link between ART procedure and imprinting disorders most convincingly BWS and less so for AS. While the magnitude of the risk remains unclear, effort should direct at optimization of ART concerning epigenetic process to forestall diseases associated with aberrant epigenetic disorders. In light of the rapidly evolving practice of ART in the sub-Saharan Africa, attention should be drawn to this concept through well organised follow-up of the offspring's.



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**REFERENCES:**

Abu-Amero, S. et al. (2008). The genetic etiology of Silver-Russell syndrome. J Med. Genet. 45: 193-199.

Anckaert, E. et al., (2009), Ammonium accumulation and use of mineral oil overlay do not alter imprinting establishment at three key imprinted genes in mouse oocytes grown and matured in a long-term follicle culture. Biol Reprod; 81:666-73.

Anckaert, E. et al.,(2009), the Unaltered imprinting establishment of key imprinted genes in mouse oocytes after in vitro follicle culture under variable follicle-stimulating hormone exposure. Int J Dev Biol; 53:541-8.

Bonakdar, M. et al., (2015), A physiology rather than a superovulated post-implantation environment can attenuate the compromising effect of assisted reproductive techniques on gene expression in developing mice embryos. : Molecular Reproduction and development. 82: 191-206.

Ceelen, M. et al., (2008), Growth and development of children born after in vitro fertilization. Fertil Steril; 90: 1662-73.

Chang, AS. Et al, (2005), Association between Beckwith-Wiedemann syndrome and assisted reproductive technology: a case series of 19 patients. Fertil Steril; 83:349-54.

Chiba, H. et al., (2013), DNA methylation error in imprinting disorders and assisted reproductive technology. Pediatric International. 55, 542-549.

Coan, PM. Et al, (2005), Imprinted genes in the placenta- a review. Placenta; 26(Suppl A): S10-20.

Cohen, J. et al. (1997), Birth of infants after transfer of enucleated donor oocyte cytoplasm into recipient eggs. Lancet, 350, 186-187.

De Waal, E. et al., (2012), Gonadotropin stimulation contributes to an increased incidence of epimutations in ICSI-derived mice. Hum Mol Genet; 21: 4460-72.

De Waal, E. et al., (2015), The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model. Human Molecular Genetics Vol. 24; 6975-6985.

DeBaun, MR. Et al., (2003), Association of in vitro fertilization with Beckwith-Wiedemann syndrome and epigenetic alterations of LIT1 and H19. American Journal of Human Genetics, 72,156-160.

Eroglu, A., and Layman, LC. (2012), Role of ART in imprinting disorders. Semin. Reprod. Med.;30 (2): 0032-1307417.

Farin, PW. Et al, (2006), Errors in development of fetuses and placentas from in vitro-produced bovine embryos. Theriogenology; 65: 178-91.

Fauque, P. (2013), ovulation induction and epigenetics anomalies. Fertility and Sterility. Vol. 99, No. 3, 0015-0282.

Fernandez-Gonzalez, R. et al., (2004), Long-term effect of in vitro culture of mouse embryos with serum on the mRNA expression of imprinting genes, development, and behavior. Proc Natl Acad U S A; 101: 5880-5.

Fernandez-Gonzalez, R. et al., (2008), Suboptimal in vitro culture conditions: An epigenetic origin of long-term health effects. Mol Reprod Dev. 74: 1149-1156.

Fortier, AL. Et al., (2008), Superovulation alters the expression of imprinted genes in the mid-gestation mouse placenta. Hum Mol Genet; 17: 1653-65.

Geuns, E. et al., (2007), Methylation analysis of KvDMR1 in human oocytes. J Med Genet; 44: 144-7.

Hajj, NT., and Haaf, T. (2013), epigenetic disturbances in vitro cultured gametes and embryos: implications for human assisted reproduction. Fertility and Sterility. Vol.99,



Print ISSN: ISSN 2053-406X, Online ISSN: ISSN 2053-4078

No. 3 0015-0282.
Hartmann, S. et al., (2006), Genetic imprinting during impaired spermatogenesis. Mol Hum Reprod:407-411.
Hawes, MS. Et al, (2002), an Ooplasmic donation in humans. The potential for epigenetic modifications. European Society of Human Reproductive and Embryology.
Hiendleder, S. et al., (2006), Tissue-specific effects of in vitro fertilization procedures on genomic cytosine methylation level in overgrown and normal sized bovine fetuses. Biol Reprod; 75: 17-23.
Hori, N. et al., (2010), Aberrant CpG methylation of the imprinting control region KvDMR1 detected in assisted reproductive technology-produced calves and pathogenesis large offspring syndrome. Amin Reprod Sci; 122:303-12.
Huang, JC. Et al, (2007), Comparison of histone modifications in vitro fertilization mouse embryos. Biochemical and Biophysical Research Communications, 354, 77-83.
Huntriss, J., and Picton, H. (2008), Epigenetic consequences of assisted reproduction and infertility on the human preimplantation embryo. Human Fertility, 11(2): 85-94.
Kobayashi et al., (2007), Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet 16: 2542-2551.
Kohda, T. (2013), Effects of embryonic manipulation and epigenetics. Journal of Human Genetics. 58, 416-420.
Laprise, SL. (2009), Implications of Epigenetics and genomic imprinting in assisted reproductive technologies. Molecular Reproduction and development; 76: 1006-1018.
Ludwig, H. (2005), Archives of Gynaecology and Obstetrics: 135 years. Arch Gynecol Obstet; 271:1-5.
Maher, ER. Et al, (2003), Beckwith-Wiedemann syndrome and assisted reproduction technology (ART). Journal of Medical Genetics, 40, 62-64.
Manipalviran, S. et al., (2009), Imprinting disorders and assisted reproductive technology. Fertility and sterility. Vol. 91, No. 2, 0015-1282.
Mann, M. et al., (2004), Selective loss of imprinting in the placenta following preimplantation development in culture. Development 131:3727-3735.
Nathan BWC and Chikondi M (2016). Infertility in Sub-Saharan Africa: A Woman's Issue for How Long? A Qualitative Review of Literature. Open Journal of Social Sciences 4:96-102
Niemann, H. et al., (2010), DNA methylation patterns reflect epigenetic reprogramming in bovine embryos. Cell Reprogram; 12:33-42.
Rancourt, RC. Et al, (2012), Methylation levels at imprinting control regions are not altered by ovulation induction or in vitro fertilization in a birth cohort. Hum Reprod; 27:2208-16.
Rivera, RM. Et al, (2008), Manipulation of mouse embryos before implantation result in aberrant expression of imprinted genes on days 9.5 of development. Hum MOL Genet; 17:1-14.
Robinson, W. et al. (2005), X- chromosome inactivation and telomere size in newborns resulting from intracytoplasmic sperm injection. Am J Med Genet A 137A(3):343-345.
Romundstad, L. et al., (2006), Effects of technology or maternal factors on perinatal outcome after assisted fertilization: A population-based cohort study. Lancet 372:737-743.
Rossignol, S. et al., (2006), The epigenetic imprinting defect of patients with Beckwith-Wiedemann syndrome born after assisted reproductive technology is not restricted to the 11p15 region. Journal of Medical Genetics, 43, 902-907.
Sato, C. et al., (2007), Assessment of human oocyte developmental competence by cumulus cell morphology and circulating hormone profile. Reprod Biomed Online; 14:49-56.



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Shah, PS. Et al, (2006), Overgrowth with a severe developmental delay following IVF/ICSI: A newly recognized syndrome? American Journal of Medical Genetics. Part A, 140, 1312-1315.

Sutcliffe, AG. Atal, (2006), Assisted reproductive therapies and imprinting disorders-a preliminary British survey. Hum Reprod; 21: 1009-11.

Tierling, S. et al., (2010), Assisted reproductive technologies do not enhance the variability of DNA methylation imprints in human. J Med Genet; 47:371-6.

Wakeling, EL. Et al, (2010), Epigenotype-phenotype corrections in Silver-Russell syndrome. J Med Genet; 47(11) :760-768.

Yanagimachi, R. (2005), Fertilization developmental initiation of oocytes by injection of spermatozoa and pre-spermatozoal cells. Ital J Anat Embryol 110: 145-150.

Yoshizawa, Y. Et al., (2010), Impaired active demethylation of the paternal genome in pronuclear-stage rat zygotes produced by in vitro fertilization or intracytoplasmic sperm injection. Mol Reprod Dev; 77:69-75.

Young, L., and Beaujean, N., (2004), DNA methylation in the preimplantation embryo: The differing stories of the mouse and sheep. Anim Reprod Sci 82-83:61-78.

Zaitseva, I. et al., (2007), Dynamics of DNA-demethylation in early mouse and rat embryos developed in vivo and in-vitro. Mol Reprod Dev; 74: 1255-61.



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