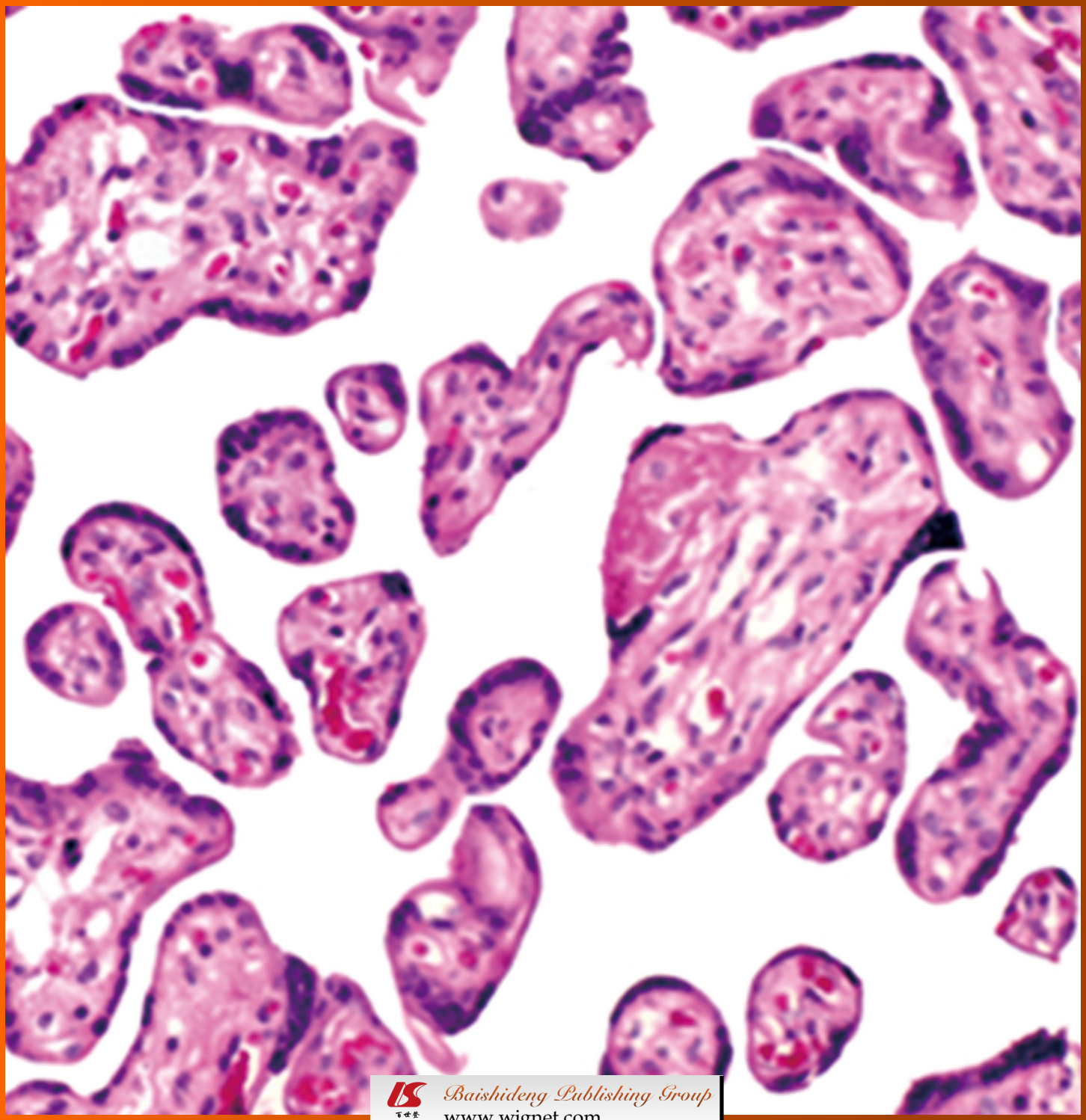
ISSN 2218-6220 (online)

World Journal of *Obstetrics and Gynecology*

World J Obstet Gynecol 2012 October 10; 1(3): 17-39



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WJOG World Journal of Obstetrics and Gynecology

A peer-reviewed, online, open-access journal of obstetrics and gynecology

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World Journal of
Obstetrics and Gynecology

Contents Bimonthly Volume 1 Number 3 October 10, 2012

World Journal of Obstetrics and Gynecology
Volume 1 Number 3 October 10, 2012

Contents

NAME OF JOURNAL
World Journal of Obstetrics and Gynecology

ISSN
ISSN 2218-6220 (online)

LAUNCH DATE
June 10, 2012

FREQUENCY
Bimonthly

EDITING
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Baishideng Publishing Group Co., Limited
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PUBLICATION DATE
October 10, 2012

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/2218-6220/g_info_20100722175812.htm.

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Obstetrics and Gynecology

Online Submissions: http://www.wjgnet.com/esps/
wjog@wjgnet.com
doi:10.5317/wjog.v1.i3.17

World J Obstet Gynecol 2012 October 10; 1(3): 17-19
ISSN 2218-6220 (online)

EDITORIAL

Prevention and not merely prediction of preterm labor and delivery

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Received: April 29, 2012 Revised: August 29, 2012
Accepted: September 12, 2012
Published online: October 10, 2012

Key words: Preterm labor; Cervical length; Prevention; Prematurity; Progesterone

Peer reviewer: Wendy Koster, MD, PhD, Department Women and Baby, University Medical Center Utrecht, Lundlaan 6, Room KE 04.123.1, PO Box 85090, 3508 AB Utrecht, The Netherlands

Jacquemyn Y. Prevention and not merely prediction of preterm labor and delivery. *World J Obstet Gynecol* 2012; 1(3): 17-19 Available from: URL: http://www.wjgnet.com/2218-6220/full/v1/i3/17.htm DOI: http://dx.doi.org/10.5317/wjog.v1.i3.17

Abstract

Different methods have been proposed to screen for preterm labor and delivery; most of these aim to predict the risk that preterm delivery is going to take place. However, interesting though this knowledge might be, knowing the future is of no use when no changes can be made. Recent publications have suggested new and exciting modalities to actually diminish the frequency of preterm birth in patients selected by transvaginal cervical length measurement; these modalities include vaginal progesterone and vaginal pessaries. Although promising, many questions remain to be answered; not least about the long term outcome for both neonates and mothers, but also on the eventual introduction of such strategies to the general obstetric population. One of the main problems that urgently needs clarification is how we are going to offer this best of medicine to those needing it most: deprived and socially isolated women who have the highest risk for preterm labor and delivery, probably not due to any congenital cervical problems, but to a combination of environmental, microbiological and social factors, including transgenerational poverty and deprivation.

Spontaneous preterm delivery is the major factor contributing to neonatal mortality and morbidity and can result in life long lasting health problems for the early born child. Optimizing neonatal care, preparing the fetus with corticosteroids and/or magnesium sulfate and trying to postpone delivery with tocolytics are all considered a form of "palliative" care once the disaster of spontaneous preterm delivery seems inevitable, but actually the success rate of these interventions is moderate at the best[1]. Over the years, a multitude of publications have been produced on methods to predict preterm delivery; from a patient's history (a previous preterm birth is a strong predictor but are we really interested in this as the majority of preterm deliveries in the western world are in primiparous women), to uterine contraction monitoring, different serum markers in maternal blood and/or saliva and, most popular, transvaginal cervical length measurement and both qualitative and quantitative detection of fetal fibronectin in cervicovaginal fluid. Logically, this is now resulting in ongoing studies to determine whether screening in low risk women is worthwhile[2]. Even then, cervical shortening and the appearance of fetal fibronectin are most probably late signs of an inflammatory response and real prevention ought to act before the inflammation is initiated. Romero coined the term "fetal inflammatory response syndrome" or FIRS[3] but

it is more probably not primarily a fetal but a maternal inflammatory response at the beginning of the chain of events resulting in preterm labor and delivery. As we still do not completely understand what is happening in normal term labor, it is hard to discover the pathological changes that lead to preterm labor. Cervical ripening and myometrial contraction also occur in term labor, accompanied by inflammatory changes, and actually, during the whole of normal pregnancy, any parameter used to express "inflammation" seems to be activated. Modulating inflammation and immune activation promises to be one of the ways to really prevent preterm labor[4] but unluckily, the most straightforward method for this, using anti-inflammatory drugs such as indomethacin, were quite successful in postponing delivery but at the expense of major fetal/neonatal side effects. It was hoped that simple interventions such as screening for asymptomatic bacteriuria and periodontal disease would significantly lower the incidence of preterm delivery. These promises have not been kept: data on the effect of treatment for asymptomatic bacteriuria are scarce and more recent findings seem to find minimal, if any, effect[5]; a meta-analysis on treating periodontal disease did not find a reduction in preterm delivery[6]. Much work has been done on bacterial vaginosis and abnormal vaginal flora (whatever that may be) and treatment in early pregnancy (treating after 20 wk is too late as the inevitable chain of events has already begun) and clindamycin seems to reduce the risk of spontaneous preterm birth at less than 37 wk but not at less than 33 wk; once again, a moderate effect at best[7]. If one tries to look at the clinical picture in a more global way, these poor results from our intervention should not be surprising: not only are we treating blindly without really understanding what we are shooting at, but all these interventions have let us lose sight of the pregnant woman as a whole in her environment. Just imagine the patient with periodontal disease: in most cases you will find that it is not just a few million bacteria in the periodontal space but it is a lack of dental and personal hygiene, often associated with smoking, unhealthy food habits and lack of access to good dental (and general health) care. Treating teeth, or urine or vaginal flora, will never compensate for social deprivation, an insecure financial situation and a health system failing to provide care to the poorest. No wonder that dedicated antenatal clinics for women with a high risk for preterm birth are not a success when we do not know what we are treating and when you do not reach the real high risk group (unplanned pregnancies in "inflammatory", meaning untreated, women), no change can be expected[8]. The real care, as with all primary prevention in medicine, should not be at a central, far away, highly specialized clinic but at the local level.

The history of the search for the prevention of preterm labor is full of promising and finally failing interventions, such as calcium and magnesium supplementation[9]. We do have a few little successes, such as abdominal cerclage in those with no or a minimal cervix[10], although the dispute about (vaginal) cerclage will probably continue for decades.

What is considered a "major advance" at the moment is the story of vaginal progesterone in the prevention of preterm labor. Vaginal progesterone can be administered in vaginal capsules, in most studies, 200 mg was used (but probably 100 mg is enough), or in a vaginal gel, in most studies 90 mg was used. Progesterone is better absorbed after application with a vaginal gel than using capsules. It has been demonstrated in a review by Rode *et al*[11] that the use of vaginal progesterone in patients with a previous preterm birth resulted in significant lowering of the percentage of women delivering before 32 wk. Once more, as with simple history taking, most preterm births occur in women giving birth for the first time.

Recently, a very well designed prospective randomized trial clearly demonstrated that screening women with transvaginal ultrasound and measuring cervical length at 19 to 23 wk, followed by vaginal progesterone gel if the cervix is between 10 and 20 mm, results in almost halving the number of preterm births[12], which confirms previous data using vaginal progesterone tablets in women with a cervix less than 15 mm[13]. This is really good news but leaves us with some questions too: Does it improve the neonatal outcome in the long term? What if the cervix is less than 10 mm? Why does it work in only half of cases and, most importantly, how will we reach those deprived high risk women and offer them a vaginal ultrasound at 19 to 23 wk, realizing that preterm delivery is more frequent in late bookings?

REFERENCES

1 **Hubinont C**, Debieve F. Prevention of preterm labour: 2011 update on tocolysis. *J Pregnancy* 2011; **2011**: 941057

2 **van Os MA**, van der Ven JA, Kleinrouweler CE, Pajkrt E, de Miranda E, van Wassenaer A, Porath M, Bossuyt PM, Bloemenkamp KW, Willekes C, Woiski M, Oudijk MA, Bilardo KM, Sikkema MJ, Duvekot JJ, Veersema D, Laudy J, Kuiper P, de Groot CJ, Mol BW, Haak MC. Preventing preterm birth with progesterone: costs and effects of screening low risk women with a singleton pregnancy for short cervical length, the Triple P study. *BMC Pregnancy Childbirth* 2011; **11**: 77

3 **Gotsch F**, Romero R, Kusanovic JP, Mazaki-Tovi S, Pineles BL, Erez O, Espinoza J, Hassan SS. The fetal inflammatory response syndrome. *Clin Obstet Gynecol* 2007; **50**: 652-683

4 **MacIntyre DA**, Sykes L, Teoh TG, Bennett PR. Prevention of preterm labour via the modulation of inflammatory pathways. *J Matern Fetal Neonatal Med* 2012; **25** Suppl 1: 17-20

5 **Lumbiganon P**, Laopaiboon M, Thinkhamrop J. Screening and treating asymptomatic bacteriuria in pregnancy. *Curr Opin Obstet Gynecol* 2010; **22**: 95-99

6 **Chambrone L**, Pannuti CM, Guglielmetti MR, Chambrone LA. Evidence grade associating periodontitis with preterm birth and/or low birth weight: II: a systematic review of randomized trials evaluating the effects of periodontal treatment. *J Clin Periodontol* 2011; **38**: 902-914

7 **Lamont RF**, Nhan-Chang CL, Sobel JD, Workowski K, Conde-Agudelo A, Romero R. Treatment of abnormal vaginal flora in early pregnancy with clindamycin for the prevention of spontaneous preterm birth: a systematic review and metaanalysis. *Am J Obstet Gynecol* 2011; **205**: 177-190

8 **Whitworth M**, Quenby S, Cockerill RO, Dowswell T. Specialised antenatal clinics for women with a pregnancy at high risk of preterm birth (excluding multiple pregnancy) to improve maternal and infant outcomes. *Cochrane Database*

Syst Rev 2011; CD006760
9 **Buppasiri P**, Lumbiganon P, Thinkhamrop J, Ngamjarus C, Laopaiboon M. Calcium supplementation (other than for preventing or treating hypertension) for improving pregnancy and infant outcomes. *Cochrane Database Syst Rev* 2011; CD007079
10 **Burger NB**, Brölmann HA, Einarsson JI, Langebrekke A, Huirne JA. Effectiveness of abdominal cerclage placed via laparotomy or laparoscopy: systematic review. *J Minim Invasive Gynecol* 2011; **18**: 696-704
11 **Rode L**, Langhoff-Roos J, Andersson C, Dinesen J, Hammerum MS, Mohapeloa H, Tabor A. Systematic review of progesterone for the prevention of preterm birth in singleton pregnancies. *Acta Obstet Gynecol Scand* 2009; **88**: 1180-1189
12 **Hassan SS**, Romero R, Vidyadhari D, Fusey S, Baxter JK, Khandelwal M, Vijayaraghavan J, Trivedi Y, Soma-Pillay P, Sambarey P, Dayal A, Potapov V, O'Brien J, Astakhov V, Yuzko O, Kinzler W, Dattel B, Sehdev H, Mazheika L, Manchulenko D, Gervasi MT, Sullivan L, Conde-Agudelo A, Phillips JA, Creasy GW. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. *Ultrasound Obstet Gynecol* 2011; **38**: 18-31
13 **da Fonseca EB**, Bittar RE, Carvalho MH, Zugaib M. Prophylactic administration of progesterone by vaginal suppository to reduce the incidence of spontaneous preterm birth in women at increased risk: a randomized placebo-controlled double-blind study. *Am J Obstet Gynecol* 2003; **188**: 419-424

S- Editor Wang JL **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Obstetrics and Gynecology

Online Submissions: http://www.wjgnet.com/esps/
wjog@wjgnet.com
doi:10.5317/wjog.v1.i3.20

World J Obstet Gynecol 2012 October 10; 1(3): 20-28
ISSN 2218-6220 (online)

REVIEW

Intrauterine growth restriction and genetic determinants - existing findings, problems, and further direction

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Received: April 18, 2012 Revised: July 6, 2012
Accepted: September 12, 2012
Published online: October 10, 2012

Abstract

Fetal growth is determined largely by the nutrient supply, placental transport function, and growth hormones. Recently, gene mutation and expression, especially of those genes associated with the proteins that are related to the fetal growth, have been reported to play an important role in the development of intrauterine growth restriction (IUGR). Fetal growth epigenetics, a new concept in fetal growth, has resulted from studies on fetal programing. This paper outlines the findings of our serial studies on IUGR, and summarizes data on IUGR animal models, placental function in transferring nutrients, cell proliferation dynamics in IUGR, and experimental treatment of IUGR. We review genetic approaches to IUGR, especially those relating to growth factor genes, angiotensinogen genes and other gene mutations. We also discuss the epigenetics of fetal growth and future study directions on fetal growth restriction. These should be valuable in elucidating the mechanisms employed by the fetus and in helping to develop interventional strategies that might prevent the development of IUGR.

Key words: Intrauterine growth restriction; Gene; Epigenetics; Placenta

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Zhang XQ. Intrauterine growth restriction and genetic determinants - existing findings, problems, and further direction. *World J Obstet Gynecol* 2012; 1(3): 20-28 Available from: URL: http://www.wjgnet.com/2218-6220/full/v1/i3/20.htm DOI: http://dx.doi.org/10.5317/wjog.v1.i3.20

INTRODUCTION

Intrauterine growth restriction (IUGR) is an important obstetric complication affecting 5% of pregnancies[1]. This condition represents an *in utero* shift from the expected pattern of fetal growth potential into reduced birth weight. This leads to increased risk for intrauterine compromise, stillbirth, preterm birth and adverse perinatal and long-term outcomes[2-5]. It is generally accepted that IUGR is associated with a poor nutrient and oxygen supply, although the specific mechanisms involved in IUGR development are mostly unknown.

Fetal growth is determined largely by the nutrient supply, which is dependent on placental transport functions. Though placental transportation depends on the concentration gradient between maternal and fetal blood, it seems that placental blood flow and the activity of specific membrane transporters play more important role[6,7]. Regardless of what the exact mechanism for IUGR might be, growth factors and their interaction with their receptors are most likely to be involved[8]. In addition to nutrition, placental function, and growth hormones, gene mutation and expression have been reported to be associated with IUGR[9-13]. Those genes associated with the proteins that are related to the fetal growth such as growth factor genes are particularly implicated. In recent years, a

new concept in fetal growth, fetal growth epigenetics, has resulted from studies on fetal programing[3,14-16].

The information in this paper outlines findings from our serial studies on IUGR and reviews the genetic approaches in recent years. This should be valuable in the elucidation of the mechanisms employed by the fetus and help in the development of interventional strategies that might prevent the development of IUGR.

EXISTING FINDINGS

IUGR animal models

Several methods have been used to establish the animal model of IUGR. These include uterine artery ligation, passive smoking, alcohol exposure, administration of L-arginine or actinomycin, and maternal malnutrition. To date, mouse, rat, guinea pig, rabbit, sheep, monkey, dog, and pig have been used to establish the IUGR model[17-19].

We have reported the establishment of an IUGR rabbit model using passive smoking and have studied the fetal and maternal plasma amino acid concentration, the blood flow of utero-placenta and changes in cell proliferation cycle in brain, liver and placenta[20,21]. In addition, the effect of treatment with histiocyte activators and vasodilators on the IUGR was also observed[7,18]. Recent studies on gene mutations have shown that specific genes might play an important role in the development of IUGR[9-16].

Active transfer of nutrients through the placenta

Although fetal plasma nutrient concentration is related to the maternal concentration, some amino acids, especially most essential amino acids, show higher concentrations in fetal plasma than in maternal plasma, proving that the absorption of amino acids from the maternal body to the fetus is an active process[22]. Maternal rabbit total plasma amino acid concentration at 28-d of pregnancy was less than that of non-pregnant rabbits. These changes were more apparent with essential amino acids. In the rabbit IUGR model, smoking could reduce the ability of the placenta to transfer nutrients from the maternal to the fetal side. It was found that the total amino acid concentration in fetus was lower in rabbits with IUGR induced by smoking than in controls. In fetal plasma, the levels of all essential amino acids, except threonine, and of nonessential amino acids including serine, glutamine, alanine, tyrosine, arginine and histidine showed the same patterns in smoking groups as in the control group[7,20].

Dynamics of cell proliferation in IUGR

Cell proliferation in the fetus is very active, especially in early embryo phase. Proliferation depends on the cell division, as measured by analyzing the cell cycle using flow cytometry. The more rapid the cell division, the shorter the G_0 and G_1 phases. In another words, lower proliferation will leave more cells in G_0 or G_1 phase, while active proliferation will result in more in S + G_2 + M phases. By analyzing the cell cycle using flow cytometry, we found that the ratio of the cells in G_0 + G_1 phases to the total cells was increased and the ratio of the cells in S + G_2 + M phases to the total cells was decreased in fetal brain, liver and placenta in IUGR rabbits induced by passive smoking. This change was particularly prominent in brain. These results suggest that the proliferation of the fetal cells and placenta were inhibited significantly, resulting in growth retardation of the fetus. This data demonstrated that the transformation from G_1 to S phases was restricted in IUGR and that cell proliferation is prevented[21,23].

Experimental treatment of IUGR

IUGR rabbits caused by passive smoking were treated with histiocyte activators (include ATP, Co-A, and Cyt-C) and vasodilators (InjectioSalviaeMoltiorrhizaeComposita). The results showed that the vasodilators could increase the uteroplacental blood flow by about 35% and induced some progress in fetal development, as indicated by a 9.8% increase in fetal body weight. The fetal plasma amino acid concentration was also higher than that of the control group. It seems that the histiocyte activators were more effective in promoting fetal development (24.6% increase of fetal body weight) than the vasodilators. Although the effect of the histiocyte activators was not as marked as that of vasodilators in increasing uteroplacental blood flow, the amino acid concentration of fetal plasma increased more after histiocyte activator treatment than vasodilator treatment. This suggests that vasodilators could be used to treat the IUGR caused by vasospasm. Histiocyte activators appear to be better than vasodilators in increasing the nutrient transportation of the placenta[24].

IUGR AND GENE APPROACHES

Although the human genome contains about 30 000 genes only a small number of them are turned on within a particular tissue type. Cell proliferation and differentiation results from the ability of tissues to express different genes from the same basic set of genetic information stored in DNA. This small subset of genes allows the cells to produce proteins unique to their functions. Although gene expression is controlled by epigenetic modifications, the gene sequence obviously plays the most important role in the proliferation and differentiation of cells.

IUGR and growth factor genes

Several studies have demonstrated altered concentrations of human placental growth hormones (hPGH), insulin-like growth factor-I (IGF-I) and IGF banding protein (IGFBPs) in the maternal circulation and the fetal compartment in pregnancies with IUGR[25,26]. Koutsaki, evaluating the expression status of the *hPGH*, the *IGF-I*, *IGFBP-1* and *IGFBP-3* genes in placentas from human IUGR pregnancies of no apparent etiology, found that *hPGH*, *IGF-I*, *IGFBP-1* and *IGFBP-3* expression was significantly lower than that in placentas showing normal

fetal growth. However, these alterations are not known causative factors of IUGR or associated with other pathogenetic mechanisms[27].

Type I insulin-like growth factor receptor (*IGF-IR*) is widely expressed across many cell types in fetal and postnatal tissues. The activation of this receptor, after the binding of secreted *IGF-I* and *IGF-IR* promotes cell differentiation and proliferation. An association has been found between IGF-*IR* gene mutations and low birth weight[28]. *IGF-IR* gene anomalies such as heterozygous *IGF-IR* mutation or insufficiency of the *IGF-IR* gene were found in patients presenting with low birth weight and birth height[29,30]. This phenotype is associated with family history of low birth weight and a normal or increased *IGF-I* level and/or a normal or increased GH response to GH stimulation test[31,32]. However, such patients show less response to GH treatment than common small for gestational age short-stature patients[33].

Choi *et al*[34] reported a family with both a novel heterozygous mutation of the *IGF-1R* gene and a segmental deletion encompassing the entire *IGF-1R*, resulting in *IGF-I* resistance and leading to IUGR and postnatal growth failure. *In vitro* studies of fibroblasts from subjects carrying the gene clearly demonstrated reduced *IGF-1R* expression and subsequent *IGF-I* resistance, as assessed by *IGF-1R* phosphorylation and post-receptor signal transduction. This indicates that *IGF-1R* mutations should be considered in the differential diagnosis of familial IUGR patients with persistent short stature[34]. Umbers found that material inflammation of the placenta could disturb the *IGF* expression and cause IUGR[35].

Experiments in animals demonstrated the importance of *IGF* in the regulation of both intrauterine and postnatal growth. Baker reported that isolated inactivation of *IGF-I* resulted in restrictions in fetal development (40% delay compared with wild type mice) and Liu found that postnatal growth was further impaired, reaching only 30% of that in normal mice[36,37]. Knockout of both *IGF-I* and *IGF-II* or knockout of both *IGF-II* and *IGF-I* receptors resulted in severe growth retardation (Figure 1)[38]. These experiments clearly demonstrated that *IGF-I* is a major regulator of both pre- and postnatal growth. Several authors reporting *IGF-IR* gene mutations observed effects on birth weight, height, serum *IGF-I* and additional complications (Table 1).

Table 1 *IGF-I* receptor mutations

Gene mutation	Birth weight (SD)	Birth height (SD)	Complications
Arg108Gln/ Lys115Asn	-3.5	-4.8	Microcephaly, abnormal speech[29]
Arg59Ter	-3.5	-3.0	Microcephaly, Delay in speech[29]
Arg709Gln	-1.5	-2.6	Mental retardation[28]
Gly1050Lys	-2.1	-4.0	Insulin resistance[32]
Arg281Gln	-3.1	-5.0	Decreased cell proliferation[31]
Val599Glu	-2.3	-2.1	Developmental delay[33]
Gly1125Ala	-1.8	-3.6	Microcephaly, clinodactyly, delayed menarche, diabetes mellitus[30]

Table 2 Maternal AGT Thr235 genotypes[40]

Groups	No.	Genotype			*P* value
		MM (%)	MT (%)	TT (%)	
Control	400	170 (42.5)	158 (39.5)	72 (18.0)	
IUGR	174	33 (19.0)	66 (37.9)	75 (43.1)	< 0.001
Preeclampsia + IUGR	60	11 (18.3)	24 (40.0)	25 (41.7)	< 0.001
Placental abruption	62	9 (14.5)	27 (43.5)	26 (41.9)	< 0.001

IUGR: Intrauterine growth restriction.

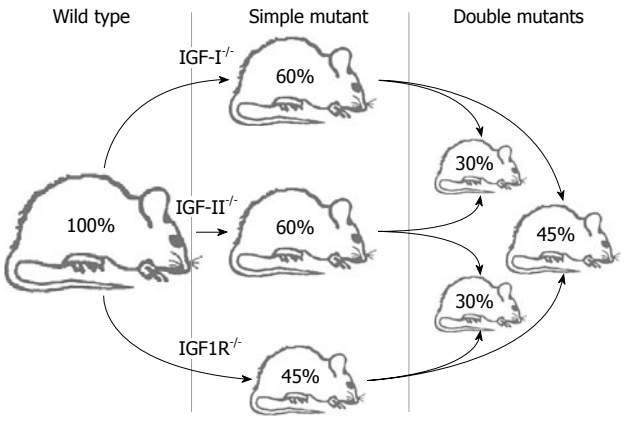

Figure 1 Effects of disruption of the insulin-like growth factor system on fetal growth in mice, expressed as a percentage of normal body weight[38]. IGF: Insulin-like growth factor.

IUGR and angiotensinogen gene

IUGR has been associated with insufficient placental circulation, which may result from failed maternal physiologic changes such as abnormal spiral artery remodeling and reduced maternal blood volume. Morgan reported that spiral artery remodeling might be related to the angiotensinogen gene[39]. We compared maternal blood DNA in 174 patients with IUGR, 62 patients with placental abruption, and 60 patients with both preeclampsia compared with a control group comprising 400 consecutive cases of women with term pregnancies and infants with birth weight between the 5th and 95th percentiles. We also examined 162 DNA samples from fetal blood with IUGR and 240 normal fetuses from control group for the Thr235 polymorphism[40] (Tables 2 and 3).

According to the AGT genotyping results using real time PCR, angiotensinogen genotypes were divided into three groups: MM (homozygous for angiotensinogen Met235 allele), TT (homozygous for angiotensinogen Thr235 allele), and MT (heterozygous). It has been demonstrated that maternal and fetal angiotensinogen Thr235 genotypes are associated with an increased risk of IUGR[40]. The angiotensinogen Thr235 allele may

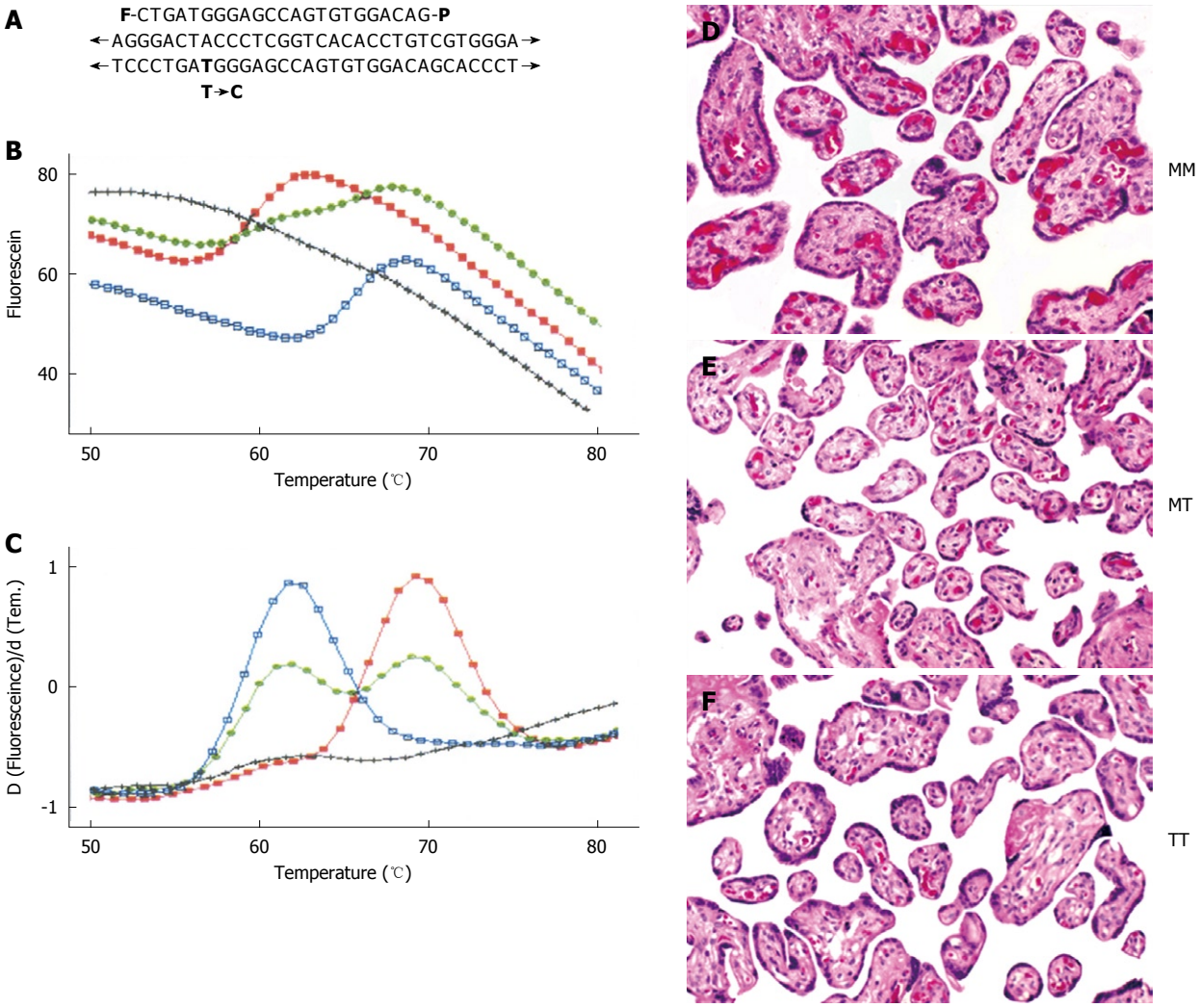

Figure 2 Real-time polymerase chain reaction for angiotensinogen M235T genotyping with a single labeled fluorescein probe[42]. A: 5'-fluorescein-labeled 23-mer (blocked at the 3'-end with phosphate) homologous to the wild-type sequence, covered the polymorphic site with the fluorescein opposite two genes; B: The probe was included in the PCR amplification mixture with primers. Melting curve data are presented as fluorescein *vs* temperature; C: Curves for homozygous wild type (peak at 69.4oC), homozygous mutant type (peak at 63.54oC), heterozygous (two peaks at 63.54oC and 69.44oC respectively) and no-template control are shown; D: Placental cross section HE staining from AGT MM, MT, and TT placentas. Quantitative analysis of the placenta indicated that the villus area (red) and capillary area (bright red) in smoking-induced intrauterine growth restriction were significantly decreased.

Table 3 AGT Thr235 alleles frequencies analysis[40]

Groups	AGT genotype		T Allele Freq.	*P* value
	Met235 alleles	Thr235 alleles		
Maternal DNA				
Control (400)	498	302	0.378	
IUGR (174)	140	208	0.598	< 0.001
Preeclampsia + IUGR (60)	44	76	0.633	< 0.001
Placental abruption (62)	45	79	0.637	< 0.001
Fetal DNA				
Control (240)	298	182	0.379	
IUGR (160)	131	189	0.591	< 0.001

IUGR: Intrauterine growth restriction.

predispose women to deliver growth-restricted fetuses. Further studies have shown that mutation of angiotensinogen gene Thr235 is also a risk factor for preeclampsia and placental abruption[41] (Table 2). In addition, quantitative analysis of the placenta indicated that the villus area was decreased significantly in the IUGR group induced by smoking exposure. The capillary area in the villus was also significantly less in IUGR groups. There were also some important changes observed under the electronic microscope, such as the retardation and reduced number of microvilli, fatty degeneration and mitochondrial swelling of the syncytial cells[42]. Studies on human placenta showed that villus volume and capillary area in IUGR were significantly decreased (Figure 2 and Table 4).

Other gene mutations

It has been reported in many publications that certain gene mutations are associated with IUGR (Table 3). Pericentric inversion of chromosome 6 causes haploinsufficiency of the *CDK19* gene resulting in microcephaly, congenital bilateral falciform retinal folds, nys-

Table 4 AGT genotype and placental findings[42]

AGT genotype	MM	MT	TT
No. of placentas	8	13	14
Clinical findings			
Maternal age (yr)	26.0 ± 4.7	26.6 ± 6.6	29.8 ± 6.5
Gestational age (wk)	36.2 ± 4.5	36.6 ± 1.9	36.7 ± 3.1
Fetal birth wt. (g)	2730 ± 967	2642 ± 541	2620 ± 535
Placental quantitative findings:			
Number of villi (mm^2)	155.3 ± 14.1	145.8 ± 28.0	146.3 ± 27.7
Villous CS area (μm^2/villous)	4422.2 ± 550.0	4400.9 ± 813.5	4248.6 ± 1191.9
Villous volume/1 cm^3 placenta (cm^3)	0.668 ± 0.034	0.626 ± 0.022[b]	0.587 ± 0.059[b,c]
Capillary volume/1 cm^3 placenta (cm^3)	0.131 ± 0.029	0.107 ± 0.034	0.070 ± 0.030[b]
Intervillous volume/1 cm^3 placenta (cm^3)	0.332 ± 0.034	0.374 ± 0.022[b]	0.413 ± 0.059[b,c]
Placental quantitative analysis:			
Volume of trimmed placenta (cm^3)	324.8 ± 128.0	355.2 ± 80.3	374.1 ± 70.2
Villous total volume per placenta (cm^3)	215.8 ± 81.8	222.1 ± 50.7	219.9 ± 52.6
Intervillous space per placenta (cm^3)	108.9 ± 47.8	133.1 ± 31.1	154.2 ± 38.6[a]
Villous capillary volume per placenta (cm^3)	45.1 ± 27.4	41.4 ± 23.2	26.6 ± 14.4[a]
Percentage of villous capillary volume (%)	19.81 ± 5.12	17.41 ± 7.3	12.06 ± 5.45[a]
Villous surface area per placenta (m^2)	9.029 ± 3.285	9.560 ± 2.031	10.370 ± 2.725
Villous surface per 1 g plac. Villi (cm^2)	390.6 ± 35.0	401.6 ± 40.5	438.6 ± 73.8

[a]$P < 0.05$, [b]$P < 0.01$ *vs* MM; [c]$P < 0.05$ *vs* MT.

tagmus, and mental retardation[9]. Chromosome 1p32-p31-deletion syndrome and haplo-insufficiency of the *NFIA* gene may present as ventriculomegaly, corpus callosum hypogenesis, abnormal external genitalia, and IUGR in the third trimester[10]. Smigiel reported on two brothers affected with restrictive dermopathy, who died in the neonatal period[43]. Molecular analyses were performed in the second child, for whom biological material was available, and on both parents. Compound heterozygous frameshifting mutations were identified in exon 1 (c.50delA) and exon 5 (c.584_585delAT) of the *ZMPSTE24* gene. The autosomal recessive inheritance was confirmed by genomic analysis of the parents. Partial loss of Ascl2 function affects all three layers of the mature placenta and causes IUGR[44]. The expression of *c-fos* is critical in the oxidative stress pathway. In fetal alcohol syndrome in mouse, it was shown that alcohol administration during pregnancy results in differential gene expression in the stress signal pathway, particularly in *c-fos*. *C-fos* expression in the decidua increases from 6 to 24 h after alcohol injection, but does not change in the embryo, which may contribute to alcohol-induced damage in fetal alcohol syndrome (Table 5)[45].

Epigenetics of fetal growth

Mechanisms leading to the attenuation of fetal birth weight and adverse pregnancy outcomes are complex. Many studies have begun to focus, not only on the contribution of maternal and fetal genes to phenotypic outcome, but also on epigenetic changes associated with fetal growth[46].

Imprinted genes have a central role in the development and function of the placenta and have been implicated in a variety of disorders affecting fetal growth[14-16,47,48]. Gene inactivation studies in mice and chromosomal rearrangements in humans have demonstrated that many of these imprinted genes play key roles in placental development and function as well as in fetal growth. Those studies have also demonstrated that imprinted genes act in a complex manner at many levels to affect the energy balance between the mother and fetus. More recent studies also support a role of imprinted genes in a compensatory response to reduced fetal growth in humans.

During early embryonic development, the first stage of embryo differentiation establishes two cell lineages. These are the inner cell mass that forms all the tissues of the adult, and the trophectoderm that eventually produces placental structures. Generally speaking, the inner cell mass becomes gene hypermethylated and the trophectoderm becomes gene hypomethylated. These methylation patterns may be preserved throughout the whole pregnancy period[49,50]. Mouse and human models suggest the epigenetic regulation of fetal growth may also play a significant role through the placental imprinted genes.

Lambertini *et al*[48] investigated the differential methylation status of the imprinted genome to gain insights into the importance of the epigenetic regulation of these genes in fetal development by comparing IUGR with normal placentas. They found that differential methylation showed a highly significant correlation with gene length. The data also suggests that differential methylation changes in growth-restricted placentas occur throughout the genomic regions encompassing genes actively expressed in the placenta. Kumar studied 11 *IGF* related genes and found upregulated *ZNF127* gene expression and down regulated *PHLDA2* gene expression. This change confirmed an increased placental expression of growth-promoting imprinted genes and decreased expression of growth-suppressive imprinted genes with advancing gestational age. These changes in placental gene expression could potentially explain accelerated fetal growth seen in the third trimester[51]. McMinn presented

Table 5 Gene and fetal growth

Gene	Gene mutation/expression	Phneotypic effects/complications
CDK19	Chromosome breakpoints in 6p12.1 and 6q21	Microcephaly, congenital bilateral falciform retinal folds, nystagmus, and mental retardation[9]
NFIA	Chromosome 1p32-p31 deletion syndrome	Ventriculomegaly, corpus callosum hypogenesis, abnormal external genitalia, and intrauterine growth restriction in the third trimester[44]
IGF1R	Novel c.420del mutation in exon 2 of the *IGF1R* gene	Reduced *IGF1R* expression and represents haploinsufficiency of the *IGF1R* gene. IUGR and neonatal growth retardation[10]
hPGH, IGF-I, IGFBP-1	Decreased expression	Decreased expression is associated with IUGR[27]
c-fos	Decreased expression	Fetal alcohol syndrome[45]
11b-HSD2	Glucocorticoid metabolism	Under expression cause IUGR, small placenta[55]
GSTP1	Glutathione transferase enzymes pathway	Fetal growth and neonatal growth[56]
ZMPSTE24	Fetal growth	IUGR, dermopathy, neonatal death[43]
Ascl2	Placenta development	Three layers malformation, IUGR[44]
TFRC	Transferrin receptor function	IUGR[13]
DIO3	Type 3 deiodinase	Highly expressed in placenta and fetus. IUGR and hypothyroidism[47]
DLK1	Growth promoter	Expressed in placental villi. Methylation defects associated with IUGR[16]
HYMAI	Non-coding RNA	Transient neonatal diabetes and IUGR[57]
IGF2	Growth Factor	Placental and fetal growth restriction[58]
KCNQ1OT1	Non-coding RNA	Control placental Kcnq1 domain. Involved in Beckwith-Wiedemann syndrome[59]
MAGEL2/NDNL1	Similarity to NDN	Neonatal growth retardation, alter metabolism[60]
MEST	Neuronal differentiation	Fetal growth restriction, smaller placentas[52]
PEG10	Retrotransposon-derivedgene	Severe growth retardation, absence of spongiotrophoblast layer, embryonic lethality[61]
PEG3	Inhibits WNT-signalling	Placental and fetal growth restriction and abnormal maternal behavior[62]
PLAGL1 Zac1	Tumor suppressor	Skeletal defects, neonatal lethality, IUGR, and disrupted transactivation of Igf2[63]
SFRP2	WNT signaling	Reduction in vitro of extra villous trophoblast invasion[64]
HBII-85/PWScr	C/D Box small RNA	Implicated in Prader Willi, Postnatal growth retardation[65]

IUGR: Intrauterine growth restriction.

an excellent example of the complexity of the imprint gene in placenta[52]. He showed that a small group of imprinted genes (*PHLDA2*, *MEST*, *MEG3*, *GATM*, *GNAS* and *PLAGL1*) and an additional 400 genes were affected in IUGR placentas.

Many studies have reported changes in imprinted gene expression and methylation levels in response to IUGR. These include reports of abnormal methylation in multiple imprinted loci[16], IUGR placental methylation decrease in the IGF2/H19 locus[53], and differential expression in multiple imprinted genes in IUGR placentas[54].

PROBLEMS AND FUTURE DIRECTION

IUGR animal models and clinical IUGR

IUGR animal models have been established by several methods. Most of the methods affect nutrients, placental function, or maternal fetal circulation although some may affect gene expression. These IUGR models have provided valuable simulations allowing study human IUGR, especially the IUGR resulting from nutritional factors, placental function, or toxic materials. However, no animal IUGR model induced by gene mutation or altered gene expression, simulating human IUGR related to specific genes has been reported. To date, the development of gene engineering and transgenic techniques have made it possible to produce specific gene mutation/expression altered IUGR animal models. This kind of animal model will provide a direct and definite method to study the specific genes involved in IUGR.

Single gene study and many genes related to IUGR

A concern of the present studies is that only limited numbers of genes have been studied among the thousands genes that are expressed in placenta, while many genes are in fact associated with fetal growth. Evaluation of their important roles in fetal growth still requires the accumulation of multiple collections of data on the mutation, phenotype, epigenetics, and metabolomics associated with fetal growth characteristics. Specifically, the pathways involved in the mutation or epigenetics resulting in IUGR phenotype are mostly unknown. Basic study of the pathways involving these genes may help us to understand why and how the phenomenon occurs at the molecular level.

Role of genes in IUGR

Although it seems simple to define the diagnosis of IUGR as an estimated fetal weight falling below the 10th percentile of normal body weight, that definition also captures births that are part of the normal variation in a population. Clinical diagnostic criteria are based on the fetal growth curve and umbilical blood velocity abnormalities. However, IUGR is still an extremely complex phenotype to dissect because of the many factors involved, maternal, fetal, placental, and environmental. Al-

most all recent gene expression studies are based on small number of samples. It is unrealistic to expect to find one or a few genes responsible for causing IUGR using this approach. Most gene expression studies have found genes that are dysregulated in IUGR. In addition, most of these genes, such as *IGF-I*, *IGFBP1*, corticotropin-releasing hormone, are reported to be related to the regulation of cell division and proliferation. We are still unable to to determine whether this is a compensatory response to restricted fetal restricted growth or a the factors which induces fetal growth restriction. The use of transgenic engineering technique in animals to study some specific gene may provide an ideal model to study the phenotype related to these genes in human clinical subjects.

Gene promoting and IUGR

Some specific imprinted genes are related to IUGR. These genes can be separated into two categories: genes participating in reducing fetal growth, and those which increase fetal growth as a compensatory response when it is sensed that the fetus is at risk. The evidence supporting mutations in imprinted genes that negatively influence fetal growth is summarized in the table and genes which the positively influence fetal growth are also indicated. We may expect that reducing expression of the negative genes and increase the expression of the positive genes would result in fetal growth. However, there is still a long way to go from basic animal model studies to reach clinical applications using gene-regulating techniques.

REFERENCES

1 **Romo A**, Carceller R, Tobajas J. Intrauterine growth retardation (IUGR): epidemiology and etiology. *Pediatr Endocrinol Rev* 2009; **6** Suppl 3: 332-336
2 **Pallotto EK**, Kilbride HW. Perinatal outcome and later implications of intrauterine growth restriction. *Clin Obstet Gynecol* 2006; **49**: 257-269
3 **Jones JE**, Jurgens JA, Evans SA, Ennis RC, Villar VA, Jose PA. Mechanisms of fetal programming in hypertension. *Int J Pediatr* 2012; **2012**: 584831
4 **Uma R**, Forsyth JS, Struthers AD, Fraser CG, Godfrey V, Murphy DJ. Polymorphisms of the angiotensin converting enzyme gene in relation to intrauterine growth restriction. *Acta Obstet Gynecol Scand* 2010; **89**: 1197-1201
5 **Kensara OA**, Wootton SA, Phillips DI, Patel M, Jackson AA, Elia M. Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen. *Am J Clin Nutr* 2005; **82**: 980-987
6 **van Meer H**, van Straten EM, Baller JF, van Dijk TH, Plösch T, Kuipers F, Verkade HJ. The effects of intrauterine malnutrition on maternal-fetal cholesterol transport and fetal lipid synthesis in mice. *Pediatr Res* 2010; **68**: 10-15
7 **Battaglia FC**. Placental transport: a function of permeability and perfusion. *Am J Clin Nutr* 2007; **85**: 591S-597S
8 **Randhawa R**, Cohen P. The role of the insulin-like growth factor system in prenatal growth. *Mol Genet Metab* 2005; **86**: 84-90
9 **Mukhopadhyay A**, Kramer JM, Merkx G, Lugtenberg D, Smeets DF, Oortveld MA, Blokland EA, Agrawal J, Schenck A, van Bokhoven H, Huys E, Schoenmakers EF, van Kessel AG, van Nouhuys CE, Cremers FP. CDK19 is disrupted in a female patient with bilateral congenital retinal folds, microcephaly and mild mental retardation. *Hum Genet* 2010; **128**: 281-291
10 **Chen CP**, Su YN, Chen YY, Chern SR, Liu YP, Wu PC, Lee CC, Chen YT, Wang W. Chromosome 1p32-p31 deletion syndrome: prenatal diagnosis by array comparative genomic hybridization using uncultured amniocytes and association with NFIA haploinsufficiency, ventriculomegaly, corpus callosum hypogenesis, abnormal external genitalia, and intrauterine growth restriction. *Taiwan J Obstet Gynecol* 2011; **50**: 345-352
11 Börzsönyi B, Demendi C, Rigó J Jr, Szentpéteri I, Rab A, Joó JG. The regulation of apoptosis in intrauterine growth restriction: a study of Bcl-2 and Bax gene expression in human placenta. J Matern Fetal Neonatal Med 2012; Epub ahead of print
12 **Chelbi ST**, Wilson ML, Veillard AC, Ingles SA, Zhang J, Mondon F, Gascoin-Lachambre G, Doridot L, Mignot TM, Rebourcet R, Carbonne B, Concordet JP, Barbaux S, Vaiman D. Genetic and epigenetic mechanisms collaborate to control SERPINA3 expression and its association with placental diseases. *Hum Mol Genet* 2012; **21**: 1968-1978
13 **Mandò C**, Tabano S, Colapietro P, Pileri P, Colleoni F, Avagliano L, Doi P, Bulfamante G, Miozzo M, Cetin I. Transferrin receptor gene and protein expression and localization in human IUGR and normal term placentas. *Placenta* 2011; **32**: 44-50
14 **He H**, Liyanarachchi S, Akagi K, Nagy R, Li J, Dietrich RC, Li W, Sebastian N, Wen B, Xin B, Singh J, Yan P, Alder H, Haan E, Wieczorek D, Albrecht B, Puffenberger E, Wang H, Westman JA, Padgett RA, Symer DE, de la Chapelle A. Mutations in U4atac snRNA, a component of the minor spliceosome, in the developmental disorder MOPD I. *Science* 2011; **332**: 238-240
15 **Edery P**, Marcaillou C, Sahbatou M, Labalme A, Chastang J, Touraine R, Tubacher E, Senni F, Bober MB, Nampoothiri S, Jouk PS, Steichen E, Berland S, Toutain A, Wise CA, Sanlaville D, Rousseau F, Clerget-Darpoux F, Leutenegger AL. Association of TALS developmental disorder with defect in minor splicing component U4atac snRNA. *Science* 2011; **332**: 240-243
16 **Turner CL**, Mackay DM, Callaway JL, Docherty LE, Poole RL, Bullman H, Lever M, Castle BM, Kivuva EC, Turnpenny PD, Mehta SG, Mansour S, Wakeling EL, Mathew V, Madden J, Davies JH, Temple IK. Methylation analysis of 79 patients with growth restriction reveals novel patterns of methylation change at imprinted loci. *Eur J Hum Genet* 2010; **18**: 648-655
17 **Vogt E**, Ng AK, Rote NS. A model for the antiphospholipid antibody syndrome: monoclonal antiphosphatidylserine antibody induces intrauterine growth restriction in mice. *Am J Obstet Gynecol* 1996; **174**: 700-707
18 **Zhang XQ**, Yan JH, Hong SY, Lin QD, Zhao AM, Huang LJ. [Establishment of intrauterine growth restriction using passive smoking]. *Shanghai Dier Yike Daxue Xuebao* 1993; **13**: 314-317
19 **Hayashi TT**, Dorko ME. A rat model for the study of intrauterine growth retardation. *Am J Obstet Gynecol* 1988; **158**: 1203-1207
20 **Zhang XQ**. [Assay of plasma amino acid concentration in maternal and growth-retarded fetuses caused by passive smoking in rabbits]. *Zhonghua Fuchanke Zazhi* 1993; **28**: 582-585, 633
21 **Zhang XQ**. [The change in dynamics of cell proliferation cycle by passive smoking in fetal rabbit]. *Zhonghua Fuchanke Zazhi* 1993; **28**: 720-722, 759
22 **Zhang XQ**, Yan JH. [Plasma amino acids concentration in maternal and fetal rabbits before and in late pregnancy]. *Zhongshan Yike Daxue Xuebao* 1995; **16**: 32-35
23 **Greenwood PL**, Slepetis RM, Hermanson JW, Bell AW.

Intrauterine growth retardation is associated with reduced cell cycle activity, but not myofibre number, in ovine fetal muscle. *Reprod Fertil Dev* 1999; **11**: 281-291
24 **Zhang X**, Yan J, Hong S. [An experimental study on the treatment of fetal rabbits with intrauterine growth retardation]. *Zhonghua Fuchanke Zazhi* 1996; **31**: 97-99
25 **Street ME**, Seghini P, Fieni S, Ziveri MA, Volta C, Martorana D, Viani I, Gramellini D, Bernasconi S. Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns with fetal growth restriction compared with controls. *Eur J Endocrinol* 2006; **155**: 567-574
26 **Verkauskiene R**, Beltrand J, Claris O, Chevenne D, Deghmoun S, Dorgeret S, Alison M, Gaucherand P, Sibony O, Lévy-Marchal C. Impact of fetal growth restriction on body composition and hormonal status at birth in infants of small and appropriate weight for gestational age. *Eur J Endocrinol* 2007; **157**: 605-612
27 **Koutsaki M**, Sifakis S, Zaravinos A, Koutroulakis D, Koukoura O, Spandidos DA. Decreased placental expression of hPGH, IGF-I and IGFBP-1 in pregnancies complicated by fetal growth restriction. *Growth Horm IGF Res* 2011; **21**: 31-36
28 **Kawashima Y**, Takahashi S, Kanzaki S. Familial short stature with IGF-I receptor gene anomaly. *Endocr J* 2012; **59**: 179-185
29 **Abuzzahab MJ**, Schneider A, Goddard A, Grigorescu F, Lautier C, Keller E, Kiess W, Klammt J, Kratzsch J, Osgood D, Pfäffle R, Raile K, Seidel B, Smith RJ, Chernausek SD. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. *N Engl J Med* 2003; **349**: 2211-2222
30 **Kruis T**, Klammt J, Galli-Tsinopoulou A, Wallborn T, Schlicke M, Müller E, Kratzsch J, Körner A, Odeh R, Kiess W, Pfäffle R. Heterozygous mutation within a kinase-conserved motif of the insulin-like growth factor I receptor causes intrauterine and postnatal growth retardation. *J Clin Endocrinol Metab* 2010; **95**: 1137-1142
31 **Inagaki K**, Tiulpakov A, Rubtsov P, Sverdlova P, Peterkova V, Yakar S, Terekhov S, LeRoith D. A familial insulin-like growth factor-I receptor mutant leads to short stature: clinical and biochemical characterization. *J Clin Endocrinol Metab* 2007; **92**: 1542-1548
32 **Walenkamp MJ**, van der Kamp HJ, Pereira AM, Kant SG, van Duyvenvoorde HA, Kruithof MF, Breuning MH, Romijn JA, Karperien M, Wit JM. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor I receptor. *J Clin Endocrinol Metab* 2006; **91**: 3062-3070
33 **Wallborn T**, Wüller S, Klammt J, Kruis T, Kratzsch J, Schmidt G, Schlicke M, Müller E, van de Leur HS, Kiess W, Pfäffle R. A heterozygous mutation of the insulin-like growth factor-I receptor causes retention of the nascent protein in the endoplasmic reticulum and results in intrauterine and postnatal growth retardation. *J Clin Endocrinol Metab* 2010; **95**: 2316-2324
34 **Choi JH**, Kang M, Kim GH, Hong M, Jin HY, Lee BH, Park JY, Lee SM, Seo EJ, Yoo HW. Clinical and functional characteristics of a novel heterozygous mutation of the IGF1R gene and IGF1R haploinsufficiency due to terminal 15q26.2-& gt; qter deletion in patients with intrauterine growth retardation and postnatal catch-up growth failure. *J Clin Endocrinol Metab* 2011; **96**: E130-E134
35 **Umbers AJ**, Boeuf P, Clapham C, Stanisic DI, Baiwog F, Mueller I, Siba P, King CL, Beeson JG, Glazier J, Rogerson SJ. Placental malaria-associated inflammation disturbs the insulin-like growth factor axis of fetal growth regulation. *J Infect Dis* 2011; **203**: 561-569
36 **Baker J**, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 1993; **75**: 73-82
37 **Liu JP**, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 1993; **75**: 59-72
38 **Netchine I**, Azzi S, Le Bouc Y, Savage MO. IGF1 molecular anomalies demonstrate its critical role in fetal, postnatal growth and brain development. *Best Pract Res Clin Endocrinol Metab* 2011; **25**: 181-190
39 **Morgan T**, Craven C, Lalouel JM, Ward K. Angiotensinogen Thr235 variant is associated with abnormal physiologic change of the uterine spiral arteries in first-trimester decidua. *Am J Obstet Gynecol* 1999; **180**: 95-102
40 **Zhang XQ**, Varner M, Dizon-Townson D, Song F, Ward K. A molecular variant of angiotensinogen is associated with idiopathic intrauterine growth restriction. *Obstet Gynecol* 2003; **101**: 237-242
41 **Zhang XQ**, Craven C, Nelson L, Varner MW, Ward KJ. Placental abruption is more frequent in women with the angiotensinogen Thr235 mutation. *Placenta* 2007; **28**: 616-619
42 **Zhang X**, Yan J, Hong S. [The quantitative and morphological analysis of placenta and brain in rabbits with intrauterine fetal growth retardation]. *Zhonghua Fuchanke Zazhi* 1995; **30**: 724-727
43 **Smigiel R**, Jakubiak A, Esteves-Vieira V, Szela K, Halon A, Jurek T, Lévy N, De Sandre-Giovannoli A. Novel frameshifting mutations of the ZMPSTE24 gene in two siblings affected with restrictive dermopathy and review of the mutations described in the literature. *Am J Med Genet A* 2010; **152A**: 447-452
44 **Oh-McGinnis R**, Bogutz AB, Lefebvre L. Partial loss of Ascl2 function affects all three layers of the mature placenta and causes intrauterine growth restriction. *Dev Biol* 2011; **351**: 277-286
45 **Poggi SH**, Goodwin KM, Hill JM, Brenneman DE, Tendi E, Schninelli S, Spong CY. Differential expression of c-fos in a mouse model of fetal alcohol syndrome. *Am J Obstet Gynecol* 2003; **189**: 786-789
46 **Suter M**, Abramovici A, Aagaard-Tillery K. Genetic and epigenetic influences associated with intrauterine growth restriction due to in utero tobacco exposure. *Pediatr Endocrinol Rev* 2010; **8**: 94-102
47 **Hernandez A**, Martinez ME, Fiering S, Galton VA, St Germain D. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. *J Clin Invest* 2006; **116**: 476-484
48 **Lambertini L**, Lee TL, Chan WY, Lee MJ, Diplas A, Wetmur J, Chen J. Differential methylation of imprinted genes in growth-restricted placentas. *Reprod Sci* 2011; **18**: 1111-1117
49 **Santos F**, Hendrich B, Reik W, Dean W. Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev Biol* 2002; **241**: 172-182
50 **Dean W**, Ferguson-Smith A. Genomic imprinting: mother maintains methylation marks. *Curr Biol* 2001; **11**: R527-R530
51 **Kumar N**, Leverence J, Bick D, Sampath V. Ontogeny of growth-regulating genes in the placenta. *Placenta* 2012; **33**: 94-99
52 **McMinn J**, Wei M, Schupf N, Cusmai J, Johnson EB, Smith AC, Weksberg R, Thaker HM, Tycko B. Unbalanced placental expression of imprinted genes in human intrauterine growth restriction. *Placenta* 2006; **27**: 540-549
53 **Tabano S**, Colapietro P, Cetin I, Grati FR, Zanutto S, Mandò C, Antonazzo P, Pileri P, Rossella F, Larizza L, Sirchia SM, Miozzo M. Epigenetic modulation of the IGF2/H19 imprinted domain in human embryonic and extra-embryonic compartments and its possible role in fetal growth restriction. *Epigenetics* 2010; **5**: 313-324
54 **Diplas AI**, Lambertini L, Lee MJ, Sperling R, Lee YL, Wetmur J, Chen J. Differential expression of imprinted genes in normal and IUGR human placentas. *Epigenetics* 2009; **4**: 235-240
55 **Börzsönyi B**, Demendi C, Pajor A, Rigó J, Marosi K, Agota A, Nagy ZB, Joó JG. Gene expression patterns of the 11β-hydroxysteroid dehydrogenase 2 enzyme in human placenta from intrauterine growth restriction: the role of im-

paired feto-maternal glucocorticoid metabolism. *Eur J Obstet Gynecol Reprod Biol* 2012; **161**: 12-17
56 **Slama R**, Gräbsch C, Lepeule J, Siroux V, Cyrys J, Sausenthaler S, Herbarth O, Bauer M, Borte M, Wichmann HE, Heinrich J. Maternal fine particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring birth weight. *Reprod Toxicol* 2010; **30**: 600-612
57 **Ma D**, Shield JP, Dean W, Leclerc I, Knauf C, Burcelin R R, Rutter GA, Kelsey G. Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM. *J Clin Invest* 2004; **114**: 339-348
58 **Dilworth MR**, Kusinski LC, Cowley E, Ward BS, Husain SM, Constância M, Sibley CP, Glazier JD. Placental-specific Igf2 knockout mice exhibit hypocalcemia and adaptive changes in placental calcium transport. *Proc Natl Acad Sci USA* 2010; **107**: 3894-3899
59 **Mohammad F**, Mondal T, Guseva N, Pandey GK, Kanduri C. Kcnq1ot1 noncoding RNA mediates transcriptional gene silencing by interacting with Dnmt1. *Development* 2010; **137**: 2493-2499
60 **Bischof JM**, Stewart CL, Wevrick R. Inactivation of the mouse Magel2 gene results in growth abnormalities similar to Prader-Willi syndrome. *Hum Mol Genet* 2007; **16**: 2713-2719
61 **Ono R**, Nakamura K, Inoue K, Naruse M, Usami T, Wakisaka-Saito N, Hino T, Suzuki-Migishima R, Ogonuki N, Miki H, Kohda T, Ogura A, Yokoyama M, Kaneko-Ishino T, Ishino F. Deletion of Peg10, an imprinted gene acquired from a retrotransposon, causes early embryonic lethality. *Nat Genet* 2006; **38**: 101-106
62 **Murphy SK**, Wylie AA, Jirtle RL. Imprinting of PEG3, the human homologue of a mouse gene involved in nurturing behavior. *Genomics* 2001; **71**: 110-117
63 **Varrault A**, Gueydan C, Delalbre A, Bellmann A, Houssami S, Aknin C, Severac D, Chotard L, Kahli M, Le Digarcher A, Pavlidis P, Journot L. Zac1 regulates an imprinted gene network critically involved in the control of embryonic growth. *Dev Cell* 2006; **11**: 711-722
64 **Guilleret I**, Osterheld MC, Braunschweig R, Gastineau V, Taillens S, Benhattar J. Imprinting of tumor-suppressor genes in human placenta. *Epigenetics* 2009; **4**: 62-68
65 **Skryabin BV**, Gubar LV, Seeger B, Pfeiffer J, Handel S, Robeck T, Karpova E, Rozhdestvensky TS, Brosius J. Deletion of the MBII-85 snoRNA gene cluster in mice results in postnatal growth retardation. *PLoS Genet* 2007; **3**: e235

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doi:10.5317/wjog.v1.i3.29

World J Obstet Gynecol 2012 October 10; 1(3): 29-34
ISSN 2218-6220 (online)

REVIEW

Free radicals generation in an *in vitro* fertilization setting and how to minimize them

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Received: March 23, 2012 Revised: June 20, 2012
Accepted: September 12, 2012
Published online: October 10, 2012

Abstract

Several studies report an increase in both male and female factors in infertility worldwide. In recent years there has been a tremendous increase in couples seeking assisted reproductive technology (ART) procedures in order to have children. However, the success rates of these procedures still remain very low. One of the major contributing factors to the low success rate in ART has been the damage caused by free radicals to the gametes and the developing embryo. The manipulation of gametes and embryos in an *in vitro* environment when performing assisted reproductive techniques carries the risk of exposure of these cells to supraphysiological levels of free radicals; namely, reactive oxygen species (ROS) and reactive nitrogen species. Oxidative stress can originate from the early steps of ART involving the oocyte, sperm and embryo, as well as in the endometrial environment later on following embryo transfer. The common sources of free radicals in an *in vitro* fertilization setting include the developing embryo, spermatozoa and leukocytes, semen centrifugation, oxygen partial pressure, light, culture media and cryopreservation/thawing. These free radicals are measured using different techniques, such as the cytochrome C reduction method and chemiluminescence-based techniques. Different efforts are being employed to minimize the excess generation of free radicals in the ART setting, with the aim of improving the success rate, and antioxidant supplementation has emerged as one of the viable routes. Moreover, it is very important to inform ART personnel about the sources of ROS in the laboratory so that they can stop the use of procedures that are deleterious and start to use safer procedures.

Key words: Reactive oxygen species; Oxidative stress; Assisted reproduction; Spermatozoa; Ovum; Antioxidants

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Lampiao F. Free radicals generation in an *in vitro* fertilization setting and how to minimize them. *World J Obstet Gynecol* 2012; 1(3): 29-34 Available from: URL: http://www.wjgnet.com/2218-6220/full/v1/i3/29.htm DOI: http://dx.doi.org/10.5317/wjog.v1.i3.29

INTRODUCTION

The presence of oxidant and antioxidant systems in various reproductive tissues has evoked great interest in the role of oxidative stress (OS) in human reproduction. OS is defined as an elevation of levels of various reactive oxygen species (ROS) that exceeds the body's antioxidant defenses[1]. It is generally accepted that an *in vitro* setup can never mimic the exact physiological conditions of an *in vivo* system. Multiple factors impinge on an *in vitro* fertilization (IVF) setting, leading to an increase in OS and suboptimal assisted reproductive technology (ART) outcome.

ROS are oxygen-derived molecules that act as powerful oxidants. Some of the ROS members, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl

radical (OH) (Figure 1), are formed as intermediary products in low concentrations in the male and female genital tracts[2]. ROS has the ability to react with any molecule and modify it oxidatively, resulting in structural and functional alterations[3]. They need to be neutralized by an elaborate defense system consisting of enzymes, such as catalase, superoxide dismutase and glutathione peroxidase or reductase, and numerous non-enzymatic antioxidants, such as vitamin C, vitamin E, vitamin A, pyruvate, glutathione, taurine and hypotaurine[4].

The effects of OS in an ART setting may be amplified due to the lack of physiological defense mechanisms available and the number of potential sources of ROS at play. Sperm damage induced by OS includes membrane and DNA damage. The use in ART of spermatozoa that have been damaged may result in altered oocyte and/or embryo development.

ROS can be produced either intracellularly, originating from gametes, or extracellularly, from environmental factors. A potential source of ROS in the ART media is its generation during preparation of semen due to the activation of ROS production by immature spermatozoa by centrifugation, the absence of the antioxidant-rich seminal plasma, or contamination by leukocytes. Similarly, oocytes and embryos contribute to the increase in ROS levels because of their metabolism and the lack of the protective antioxidant mechanisms present in their natural habitat[5,6]. The external environment surrounding the ART procedure also plays an important role in the development of OS. The most important external factor that may affect gamete and embryo viability *in vitro* is oxygen partial pressure (pO_2). Cells cultured *in vitro* are exposed to a relatively hypertoxic environment compared with the *in vivo* conditions. In addition to pO_2, other physicochemical environmental factors such as temperature variation may also affect gamete and embryo development *in vitro*. Therefore, the external environment in the ART procedure can be a potential source of OS. Recently, studies have indicated the upper control limit of ROS in follicular fluid beyond which viable embryo formation is not favorable[7], as well as cut-off values for predicting semen quality and fertilization outcome[8].

Reactive nitrogen species such as nitric oxide (NO) have also been shown to play a role in reproduction. The production of NO is catalyzed by a family of NO synthase (NOS) enzymes[9]. NOS is responsible for the conversion of L-arginine to NO and L-citrulline[10]. Two NOS types have been identified in human spermatozoa: NOS, similar to the constitutively expressed brain neuronal NOS, and endothelial NOS[11]. The ability of human spermatozoa to synthesize NO has been demonstrated indirectly by measuring nitrite accumulation[12] and L-[^{3}H] citrulline generation[13], or directly by means of an NO meter[11] and fluorescence activated cell sorting (FACS) analysis[14].

In vitro studies have shown contrasting results of exogenous NO yield on human sperm function, depending on the concentrations applied[15]. It has been reported that lower concentrations of NO are beneficial to human sperm function, whereas higher concentrations become detrimental[16]. This review is aimed at discussing sources of free radicals in an IVF setting and how to minimize their generation.

SOURCES OF FREE RADICALS IN AN IVF SETTING

Developing embryo

Like any other living aerobic cells, the developing embryo and the oocyte could be a major source of ROS because they use oxygen to produce energy through mitochondrial oxidative phosphorylation. This ROS is produced through several pathways, such as oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase systems[17]. Studies have reported that ROS production is increased in embryos cultured *in vitro* compared with those *in vivo*[5]. It is therefore recommended that embryo culture media should be supplemented with antioxidants that can scavenge the excess ROS that is generated by the developing embryo.

Spermatozoa and leukocytes

Spermatozoa and other cells in semen are huge sources of ROS. Morphologically abnormal and immature spermatozoa, together with the presence of leukocytes, can generate ROS in human ejaculates. The sperm generate ROS at the level of plasma membrane and mitochondria[18]. Studies have shown that human spermatozoa generate O_2^-, which spontaneously dismutates to H_2O_2[19].

In the male genital tract and the ejaculate, ROS are not only derived from the spermatozoa but can also be generated by leukocytes, which physiologically produce up to 1000 times more ROS than spermatozoa[20,21]. This high ROS production by leukocytes plays a major role in infections, inflammation and cellular defense mechanisms. Basically, the cellular mechanisms for the generation of ROS in leukocytes and spermatozoa are the same, yet in leukocytes it is a physiological necessity to release large amounts of superoxide into phagocytic vesicles during the killing action of pathogens.

Considering the extraordinary high content of polyunsaturated fatty acids in their membrane, the sperm plasma membrane is particularly susceptible to OS and the double bonds of the membrane lipids can easily be oxidized by excessive ROS levels present in the sperm cells' environment. These can either be produced in large amounts by leukocytes or the spermatozoa themselves. In the case of ROS attacking the plasma membrane lipids, a process called "lipid peroxidation" (LPO) is initiated. Ultimately, this process decreases membrane fluidity of both plasma and organelle membranes and, as a result, damages membrane function, ion gradients, receptor-mediated signal transduction, *etc.*[22]. Hence, with the loss of membrane function, spermatozoa lose the ability to function properly and therefore fertilization is impaired[23].

It is therefore advisable that patients with high leu-

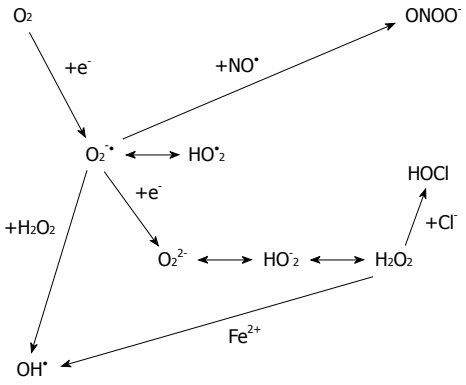

Figure 1 Derivation of reactive oxygen species from oxygen.

kocyte levels in their ejaculate (leukocytospermia) must have their spermatozoa quickly separated from the leukocytes to avoid ROS damage due to leukocyte activation. Supplementation with scavengers would also help to minimize OS.

Semen centrifugation

The removal of sperm from the seminal fluid is a very important step in the processing of semen during assisted reproductive procedures. Most sperm processing techniques employ the use of centrifugation to separate motile sperm from non-motile or dead sperm, as well as other contaminating cell debris[24]. However, sperm processing techniques that employ the use of centrifugation have their own disadvantages. Studies have shown that sperm preparation, processing and handling lead to increased free radical generation[25]. We have also shown in our previous study that centrifuging spermatozoa for more than 10 min led to an increase in ROS generation[26]. On the other hand, we demonstrated that 10 min of sperm centrifugation also increased NO generation, whereas 30 min of centrifugation led to a decrease in NO generation. The decrease in NO generation after 30 min of centrifugation was probably due to NOS enzyme down-regulation or loss. Alternatively, it could be that NO reacted with other members of the ROS family such as O_2^- to form peroxynitrite anion ($ONOO^-$) since NO has been shown to possess antioxidant abilities[27].

It is therefore recommended that sperm separation techniques should avoid using centrifugation or prolonged centrifugation in assisted reproductive technologies. The addition of ROS scavengers prior to centrifugation could also help in reducing free radical generation and therefore improving sperm recovery.

Oxygen partial pressure

The pO_2 in the laboratory environment is very different from that of the *in vivo* condition. It is higher in the *in vitro* laboratory setting; thus the higher pO_2 activates various oxidase enzyme systems in the cells and contributes to increased ROS generation[28]. Gametes and the media in the IVF setting should therefore not be exposed to high pO_2 to prevent excess ROS generation.

Light

Visible light has been reported to be another contributor that leads to increased ROS generation. Light induces photodynamic stress, leading to oxidative damage of unsaturated lipids and sterols within the membranes[29]. Studies have shown that exposing mouse embryos to transient visible light led to an increase in the generation of H_2O_2[5]. Therefore, gametes meant for use during assisted reproduction should not be exposed to direct light to avoid excess generation of free radicals.

Culture media

Different types of culture media are used in ART laboratories. These media have different ingredients which can lead to ROS generation. In some culture media there are metallic ions such as Fe^{2+} and Cu^{2+} which have the potential of accelerating ROS generation within the cell by participating in Fenton and Haber-Weiss reactions[17]. Furthermore, media additives such as serum albumin contain high levels of amine oxidase which leads to an increase in H_2O_2 production[30].

It is therefore recommended that culture media used in IVF settings should be supplemented with antioxidants. Antioxidants such as urate, isoflavones, ascorbic acid, taurine, hypotaurine, genistein and α-tocopherol can be used as culture media supplements to minimize the risk of OS and damage it causes to the gametes[4,31]. Vitamin E supplementation has also been shown to protect gametes from the LPO[32]. In order to prevent ROS generation by metallic ions found in culture media, metal chelators can be added to the media.

Cryopreservation-thawing

Many studies have reported that cryopreservation enhances LPO through ROS induced membrane lipid damage[33]. Cryopreserved sperm has been reported to be susceptible to ROS insults since they tend to lose their antioxidant defenses[34]. It is therefore recommended that improved cryopreservation protocols that optimize the use of cryoprotectants should be used to improve the quality of cryopreserved gametes.

MEASURING FREE RADICALS

There are different techniques that are employed to measure free radicals. An ideal assay for ROS detection should be sufficiently sensitive to ensure that measurements are within the linear range of the assay and well above the limits of detection. Preferably, it should be specific for one ROS, at least in physiological/pathophysiological concentrations. Its signal should be substantially inhibited by a specific scavenger of the ROS in question. It should be robust; that is, applicable to a wide variety of experimental conditions and comparable between these applications. However, ROS production can be highly localized and therefore most assays will not be able to detect ROS in all of the subcellular locations and extracellularly. Furthermore, the concentration of ROS at the site

of production could be extremely high and this almost certainly will not be reflected by measurements obtained in intact tissues, cells or in the media in which tissues are incubated. Finally, it is very unlikely that any probe will react with all of the ROS produced, meaning that any assay cannot be viewed as completely quantitative.

Cytochrome C reduction

Oxygen is a diradical, possessing two unpaired electrons. When oxygen gains an additional electron, $O_2^{\cdot-}$ is formed. Superoxide is generally considered an oxidant because in most cases it extracts an electron from another molecule, leading to formation of H_2O_2. For other molecules with higher electron potentials, $O_2^{\cdot-}$ actually donates its extra electron. This is the case for ferricytochrome c, which is reduced to ferrocytochrome c by receiving an electron from $O_2^{\cdot-}$. When ferricytochrome c is reduced, its spectrophotometric absorbance is altered in a very specific fashion. Absorbance at 550 nm is increased, whereas absorption at 540 and 560 nm remain unaltered and can serve as an isosbestic point. Unfortunately, ferricytochrome c can be directly reduced by electrons donated from enzymes and other molecules, so this change in absorbance is not specific for $O_2^{\cdot-}$. For this reason, the assay must be performed in the presence and absence of SOD and only the SOD-inhibitable signal used to calculate the amount of $O_2^{\cdot-}$ formed.

Procedure: For measurement of $O_2^{\cdot-}$, it is necessary to divide the cells in two aliquots of equal concentrations. Cells are placed in a buffer composed of (mmol/L): NaCl, 145; KCl, 4.86; NaH_2PO_4, 5.7; $CaCl_2$, 0.54; $MgSO_4$, 1.22; glucose, 5.5; deferoxamine mesylate, 0.1; and 50 μmol/L of acetylated ferricytochrome c. Another aliquot of cells is added to a similar buffer containing manganese superoxide dismutase (100 U/mL). The cells are incubated in a 96-well plate to minimize the volume and thus maximize the concentration of $O_2^{\cdot-}$ released from the cells exposed to ferricytochrome c. Acetylation of cytochrome c improves quantification and specificity for $O_2^{\cdot-}$ because it strongly inhibits direct enzymatic reduction, improves stability of the reduced cytochrome c, and has minimal effects on reaction with $O_2^{\cdot-}$[35,36]. To further ensure that any reduced ferricytochrome c is not reoxidized, catalase (125 U/mL) is added to the reaction, which removes any H_2O_2 formed. The cells are incubated in these reaction mixtures for 30 min at 37 ℃. The cells are then removed and the buffer absorbances measured at 540, 550 and 560 nm using a 96-well plate reader.

Chemiluminescence-based techniques

On exposure to $O_2^{\cdot-}$, chemiluminescent probes release a photon, which in turn can be detected by a scintillation counter or a luminometer. Because most of these compounds are cell permeable, the $O_2^{\cdot-}$ measured reflects extracellular, as well as intracellular, $O_2^{\cdot-}$ production. The most commonly used chemiluminescence technique for measurement of $O_2^{\cdot-}$ is lucigenin-enhanced chemiluminescence.

Procedure: For the assay, a Krebs/HEPES buffer containing 5 μmol/lucigenin is prepared. It has been found that the results are most consistent if this buffer is "dark adapted." The buffer is placed in either a luminometer or scintillation counter set to the out-of-coincidence mode and incubated until background counts have stabilized. The cells are then added to the reaction vial and, after 15 min of equilibration, counts are obtained every minute for the next 5 min and averaged. Counts can be expressed as chemiluminescence units.

This method has been widely used and an enormous amount of new information has been gained from its use. It is reasonably specific for $O_2^{\cdot-}$ and one does not need to routinely prepare a second sample with SOD to prove that the signal is derived from $O_2^{\cdot-}$. If one chooses to use an $O_2^{\cdot-}$ scavenger, it should be kept in mind that lucigenin penetrates cells and therefore unmodified SOD, which is not cell permeable, will not reduce the lucigenin signal completely (usually ≤ 50%). The assay is also inexpensive and can be performed using equipment that is commonly available.

Detection of intracellular H_2O_2 with DCF-DA

One of the most commonly used probes for the detection of intracellular ROS formation is 2'7'-dichlorofluorescein diacetate (DCFH-DA)[37-39]. DCFH-DA is cell permeable and after uptake it is cleaved by intracellular esterases to 2',7'-dichlorofluorescin (DCFH), trapped within the cells and oxidized to the fluorescent molecule 2',7'-dichlorofluorescein (DCF) by a variety of ROS[40]. DCFH-DA is considered a general indicator of ROS, reacting with H_2O_2, $ONOO^-$, lipid hydroperoxides and, to a lesser extent, $O_2^{\cdot-}$. Because H_2O_2 is a secondary product of $O_2^{\cdot-}$, DCF fluorescence has been used to implicate $O_2^{\cdot-}$ production.

Procedure: For *in situ* localization of H_2O_2, cells are incubated with DCFH-DA (5 μmol/L; Molecular Probes)[41] for 30 min at 37 ℃[42]. H_2O_2 detection is confirmed by simultaneously treating replicate cells with polyethylene glycol-catalase (350 U/mL). Cells incubated with vehicle can serve as negative controls. All of the images are acquired at identical settings. DCFH-DA fluorescence can also be measured using fluorescence-activated cell sorter analysis. A Becton Dickinson FACSCalibur™ analyzer can be used to quantify fluorescence at a single cell level and data analyzed by using CellQuest™. In each sample, the mean fluorescence intensity of the analyzed cells is determined after prior gating the cell population by forward and side light scatter signals, as recorded on the dot plot (Figure 2). In total, 100 000 events can be acquired but non-sperm particles and debris (located on the bottom left corner of the dot plot) are excluded by prior gating, thereby limiting undesired effects on overall fluorescence. The fluorescence signals are recorded on a frequency histogram (Figure 3A and B) using logarithmic amplification. Fluorescence data is expressed as mean fluorescence (percentage of control, control adjusted to 100%).

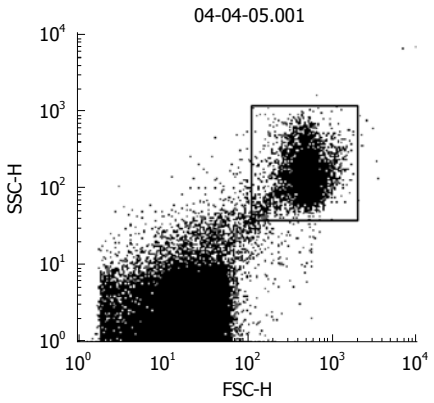

Figure 2 A dot plot of sperm cells showing the spread of the total recorded "events". Gated population (top right): sperm cells and bottom left: non-sperm particles, debris.

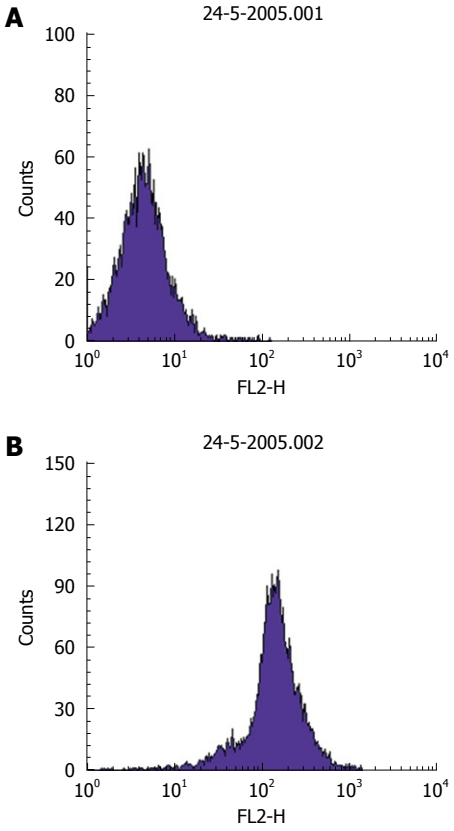

Figure 3 A representative frequency histogram showing baseline fluorescence (log) of DCFH on x-axis (A) and a shift to right depicting an increase in fluorescence intensity (B).

Detection of extracellular H_2O_2 with Amplex Red

The Amplex Red assay involves measurement of H_2O_2 by the horseradish peroxidase–catalyzed oxidation of the colorless and nonfluorescent molecule N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) to resorufin, which, when excited at 530 nm, strongly emits light at 590 nm[43].

Procedure: The cells are placed in the well of a 96-well plate and incubated with Amplex Red (10 μmol/L) and horseradish peroxidase (0.2 U/mL) for 60 min at 37 ℃ in Krebs Ringer's phosphate glucose buffer (145 mmol/L of NaCl, 5.7 mmol/L of sodium phosphate, 4.86 mmol/L of KCl, 0.54 mmol/L of $CaCl_2$, 1.22 mmol/L of $MgSO_4$ and 5.5 mmol/L of glucose) protected from light. The cells are removed from the buffer and the buffer's fluorescence is detected at 590 nm using an excitation 530 nm. Background fluorescence, determined using a reaction without cells, is subtracted from each value. H_2O_2 release, calculated using H_2O_2 standards or standard solutions of resorufin, is expressed as picomoles per concentration of cells[44]. In some experiments, SOD (40 U/mL) or catalase (200 U/mL) can be added to the cells.

CONCLUSION

OS plays a very important role in the outcome of assisted reproduction. There are several factors that lead to excess free radical generation in an ART setting; therefore, the laboratory personnel must be aware of these sources of free radicals generation. This review has outlined some of the most common factors that contribute to OS. It is also very important that routine free radical measurements should be practiced in an ART setting so that laboratory personnel can be aware of practices that lead to free radicals generation. The methods of free radical measurement summarized in this review are easy to follow, provided the equipment is available. It should also be noted that antioxidant supplementation should be part of the solution to minimize OS in an ART setting so that the success rate of assisted reproduction outcomes can be increased.

REFERENCES

1 **Agarwal A**, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003; **79**: 829-843
2 **Agarwal A**, Allamaneni SS. Role of free radicals in female reproductive diseases and assisted reproduction. *Reprod Biomed Online* 2004; **9**: 338-347
3 **Sharma RK**, Agarwal A. Role of reactive oxygen species in male infertility. *Urology* 1996; **48**: 835-850
4 **Sikka SC**. Role of oxidative stress and antioxidants in andrology and assisted reproductive technology. *J Androl* 2004; **25**: 5-18
5 **Goto Y**, Noda Y, Mori T, Nakano M. Increased generation of reactive oxygen species in embryos cultured in vitro. *Free Radic Biol Med* 1993; **15**: 69-75
6 **Nasr-Esfahani MH**, Johnson MH. How does transferrin overcome the in vitro block to development of the mouse preimplantation embryo? *J Reprod Fertil* 1992; **96**: 41-48
7 **Jana SK**, K NB, Chattopadhyay R, Chakravarty B, Chaudhury K. Upper control limit of reactive oxygen species in follicular fluid beyond which viable embryo formation is not favorable. *Reprod Toxicol* 2010; **29**: 447-451
8 **Das S**, Chattopadhyay R, Jana SK, Narendra BK, Chakraborty C, Chakravarty B, Chaudhury K. Cut-off value of reactive oxygen species for predicting semen quality and fertilization outcome. *Syst Biol Reprod Med* 2008; **54**: 47-54
9 **Thundathil J**, de Lamirande E, Gagnon C. Nitric oxide regulates the phosphorylation of the threonine-glutamine-tyrosine motif in proteins of human spermatozoa during capacitation. *Biol Reprod* 2003; **68**: 1291-1298

10 **O'Bryan MK**, Zini A, Cheng CY, Schlegel PN. Human sperm endothelial nitric oxide synthase expression: correlation with sperm motility. *Fertil Steril* 1998; **70**: 1143-1147
11 **Donnelly ET**, Lewis SE, Thompson W, Chakravarthy U. Sperm nitric oxide and motility: the effects of nitric oxide synthase stimulation and inhibition. *Mol Hum Reprod* 1997; **3**: 755-762
12 **Lewis SE**, Donnelly ET, Sterling ES, Kennedy MS, Thompson W, Chakravarthy U. Nitric oxide synthase and nitrite production in human spermatozoa: evidence that endogenous nitric oxide is beneficial to sperm motility. *Mol Hum Reprod* 1996; **2**: 873-878
13 **Revelli A**, Soldati G, Costamagna C, Pellerey O, Aldieri E, Massobrio M, Bosia A, Ghigo D. Follicular fluid proteins stimulate nitric oxide (NO) synthesis in human sperm: a possible role for NO in acrosomal reaction. *J Cell Physiol* 1999; **178**: 85-92
14 **Lampiao F**, Strijdom H, du Plessis SS. Direct nitric oxide measurement in human spermatozoa: flow cytometric analysis using the fluorescent probe, diaminofluorescein. *Int J Androl* 2006; **29**: 564-567
15 **Sengoku K**, Tamate K, Yoshida T, Takaoka Y, Miyamoto T, Ishikawa M. Effects of low concentrations of nitric oxide on the zona pellucida binding ability of human spermatozoa. *Fertil Steril* 1998; **69**: 522-527
16 **Wu TP**, Huang BM, Tsai HC, Lui MC, Liu MY. Effects of nitric oxide on human spermatozoa activity, fertilization and mouse embryonic development. *Arch Androl* 2004; **50**: 173-179
17 **Guérin P**, El Mouatassim S, Ménézo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod Update* 2001; **7**: 175-189
18 **Gavella M**, Lipovac V. NADH-dependent oxidoreductase (diaphorase) activity and isozyme pattern of sperm in infertile men. *Arch Androl* 1992; **28**: 135-141
19 **Alvarez JG**, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. *J Androl* 1987; **8**: 338-348
20 **Plante M**, de Lamirande E, Gagnon C. Reactive oxygen species released by activated neutrophils, but not by deficient spermatozoa, are sufficient to affect normal sperm motility. *Fertil Steril* 1994; **62**: 387-393
21 **de Lamirande E**, Gagnon C. Capacitation-associated production of superoxide anion by human spermatozoa. *Free Radic Biol Med* 1995; **18**: 487-495
22 **Sikka SC**, Rajasekaran M, Hellstrom WJ. Role of oxidative stress and antioxidants in male infertility. *J Androl* 1995; **16**: 464-468
23 **Riffo MS**, Parraga M. Study of the acrosome reaction and the fertilizing ability of hamster epididymal cauda spermatozoa treated with antibodies against phospholipase A2 and/or lysophosphatidylcholine. *J Exp Zool* 1996; **275**: 459-468
24 **Henkel RR**, Schill WB. Sperm preparation for ART. *Reprod Biol Endocrinol* 2003; **1**: 108
25 **Agarwal A**, Ikemoto I, Loughlin KR. Effect of sperm washing on levels of reactive oxygen species in semen. *Arch Androl* 1994; **33**: 157-162
26 **Lampiao F**, Strijdom H, du Plessis SS. Effects of sperm processing techniques involving centrifugation on nitric oxide, reactive oxygen species generation and sperm function. *Open Androl J* 2010; **2**: 1-5
27 **Oates JC**, Gilkeson GS. The biology of nitric oxide and other reactive intermediates in systemic lupus erythematosus. *Clin Immunol* 2006; **121**: 243-250
28 **Cohen J**, Gilligan A, Esposito W, Schimmel T, Dale B. Ambient air and its potential effects on conception in vitro. *Hum Reprod* 1997; **12**: 1742-1749
29 **Girotti AW**. Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *J Photochem Photobiol B* 2001; **63**: 103-113
30 **Shannon P**. Factors affecting semen preservation and conception rates in cattle. *J Reprod Fertil* 1978; **54**: 519-527
31 **Alvarez JG**, Storey BT. Taurine, hypotaurine, epinephrine and albumin inhibit lipid peroxidation in rabbit spermatozoa and protect against loss of motility. *Biol Reprod* 1983; **29**: 548-555
32 **Jain SK**, McVie R, Smith T. Vitamin E supplementation restores glutathione and malondialdehyde to normal concentrations in erythrocytes of type 1 diabetic children. *Diabetes Care* 2000; **23**: 1389-1394
33 **Alvarez JG**, Storey BT. Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a mode of sublethal cryodamage to human sperm during cryopreservation. *J Androl* 1992; **13**: 232-241
34 **Bilodeau JF**, Chatterjee S, Sirard MA, Gagnon C. Levels of antioxidant defenses are decreased in bovine spermatozoa after a cycle of freezing and thawing. *Mol Reprod Dev* 2000; **55**: 282-288
35 **Azzi A**, Montecucco C, Richter C. The use of acetylated ferricytochrome c for the detection of superoxide radicals produced in biological membranes. *Biochem Biophys Res Commun* 1975; **65**: 597-603
36 **Green TR**, Pratt KL. A reassessment of the product specificity of the NADPH: O2 oxidoreductase of human neutrophils. *Biochem Biophys Res Commun* 1987; **142**: 213-220
37 **Wang H**, Joseph JA. Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader. *Free Radic Biol Med* 1999; **27**: 612-616
38 **Kooy NW**, Royall JA, Ischiropoulos H. Oxidation of 2',7'-dichlorofluorescin by peroxynitrite. *Free Radic Res* 1997; **27**: 245-254
39 **Zulueta JJ**, Sawhney R, Yu FS, Cote CC, Hassoun PM. Intracellular generation of reactive oxygen species in endothelial cells exposed to anoxia-reoxygenation. *Am J Physiol* 1997; **272**: L897-L902
40 **Bass DA**, Parce JW, Dechatelet LR, Szejda P, Seeds MC, Thomas M. Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. *J Immunol* 1983; **130**: 1910-1917
41 **Khatri JJ**, Johnson C, Magid R, Lessner SM, Laude KM, Dikalov SI, Harrison DG, Sung HJ, Rong Y, Galis ZS. Vascular oxidant stress enhances progression and angiogenesis of experimental atheroma. *Circulation* 2004; **109**: 520-525
42 **Liu Y**, Zhao H, Li H, Kalyanaraman B, Nicolosi AC, Gutterman DD. Mitochondrial sources of H2O2 generation play a key role in flow-mediated dilation in human coronary resistance arteries. *Circ Res* 2003; **93**: 573-580
43 **Zhou M**, Diwu Z, Panchuk-Voloshina N, Haugland RP. A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases. *Anal Biochem* 1997; **253**: 162-168
44 **Weber DS**, Rocic P, Mellis AM, Laude K, Lyle AN, Harrison DG, Griendling KK. Angiotensin II-induced hypertrophy is potentiated in mice overexpressing p22phox in vascular smooth muscle. *Am J Physiol Heart Circ Physiol* 2005; **288**: H37-H42

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Online Submissions: http://www.wjgnet.com/esps/
wjog@wjgnet.com
doi:10.5317/wjog.v1.i3.35

World J Obstet Gynecol 2012 October 10; 1(3): 35-39
ISSN 2218-6220 (online)

BRIEF ARTICLE

Platinum-resistant ovarian cancer: Prematurely stopped phase Ⅱ Austrian AGO chemotherapy studies

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Received: April 6, 2012 Revised: July 9, 2012
Accepted: September 12, 2012
Published online: October 10, 2012

Abstract

AIM: To report the results of two phase Ⅱ studies of chemotherapy in patients with platinum-resistant and platinum-refractory ovarian cancer and discuss the current status of systemic therapy in this disease.

METHODS: Two subsequent Austrian Arbeitsgemeinschaft für Gynäkologische Onkologie (AGO) phase Ⅱ studies have been carried out. Patients either had platinum-refractory or platinum-resistant disease, i.e., disease progression during first line platinum-based therapy or recurrence within 6 mo following the last platinum-containing chemotherapy, respectively. In the first study, 6 cycles of irinotecan at 55 mg/m^2 and docetaxel 25 mg/m^2 were both administered on days 1, 8 and 15 of a 4 wk cycle. In the second phase Ⅱ study, either non-pegylated (PEG) liposomal doxorubicin (L-DXR) 60 mg/m^2 monotherapy on day 1 and PEG filgrastim on day 2 (arm A) or L-DXR at 50 mg/m^2 and gemcitabine (GEM) at 650 mg/m^2 on day 1 and GEM on day 8 (arm B) were administered every 4 wk. Patients in arm B received prophylactic filgrastim 5 μg/kg per day from days 3 to 6 and from days 9 to 12, respectively.

RESULTS: Response rates in studies were 14% and 17%, respectively. The progression-free survival was less than 3 mo. Diarrhea was most prevalent in patients treated with irinotecan + docetaxel, while stomatitis/mucositis occurred in a quarter of patients treated with L-DXR +/- GEM + granulocyte colony stimulating factor, respectively. Following treatment with the latter regimen, a total of 11 serious adverse events were recorded among the 12 patients included. The rate of remissions of the regimens used in these two Austrian AGO studies was low and their toxicity significant. Due to their low therapeutic index, neither of these regimens can be recommended in this heavily pretreated patient population with platinum-resistant ovarian cancer exhibiting a high tumor-associated symptom burden.

CONCLUSION: The two reported phase Ⅱ studies of the Austrian AGO in platinum-resistant disease had to be terminated prematurely due to a low therapeutic index. Treatment of this disease remains a clinical dilemma. Bevacizumab seems to be active at this late-

stage disease but may be associated with significant bowel toxicity.

Key words: Platinum-resistance; Ovarian cancer; Chemotherapy; Gemcitabine; Non-pegylated liposomal doxorubicin; Irinotecan; Docetaxel

Peer reviewer: Federico Coccolini, MD, General and Emergency Surgery Department, Ospedali Riuniti, Largo Barozzi, 1, 24126 Bergamo, Italy

Petru E, Volgger B, Bogner G, Angleitner-Boubenizek L, Deibl M, Schauer C, Reinthaller A, Wolfram G, Zeimet AG, Marth C. Platinum-resistant ovarian cancer: Prematurely stopped phase Ⅱ Austrian AGO chemotherapy studies. *World J Obstet Gynecol* 2012; 1(3): 35-39 Available from: URL: http://www.wjgnet.com/2218-6220/full/v1/i3/35.htm DOI: http://dx.doi.org/10.5317/wjog.v1.i3.35

INTRODUCTON

Ovarian cancer carries the highest mortality among all gynecological malignancies. In this disease, after an initial response to platinum and a taxane, resistance development is a significant problem. At this stage of disease, patients usually suffer from significant tumor-related symptoms and chemotherapy is only moderately active. The median survival lies between 6 and 11 mo[1,2].

In platinum-resistant ovarian cancer, monotherapy with pegylated (PEG)-liposomal doxorubicin (L-DXR), weekly paclitaxel, topotecan and gemcitabine (GEM) are the most commonly used agents[3,4]. There is an urgent need for more effective therapies able to induce a remission and thus leading to effective palliation.

Since 2005, the Austrian Arbeitsgemeinschaft für Gynäkologische Onkologie (AGO) has initiated three subsequent studies which investigated the use of combination regimens in platinum-refractory and platinum-resistant ovarian cancer. One study of topotecan and γ-interferon will be published elsewhere.

The results of the other two phase Ⅱ studies are reported here. The aim was to define the response rates, the progression-free survival and the toxicity profiles. The authors also discuss the current status of systemic therapy in platinum-resistant disease.

MATERIALS AND METHODS

Two subsequent Austrian AGO phase Ⅱ studies were carried out between 2005 and 2011 (Table 1). All patients either had platinum-refractory or platinum-resistant disease, i.e., disease progression during first-line platinum-taxane therapy or recurrence within 6 mo following the last platinum-containing chemotherapy, respectively. Patients were treated at the Departments of Obstetrics and Gynecology of the Medical Universities of Graz, Innsbruck and Vienna, as well as at the Departments of Gynecology of the Hospitals Barmherzige Schwestern in Linz, Ried and the Hospital Barmherzige Brüder, Graz and the Departments of Obstetrics and Gynecology, Wels, Neunkirchen and Kufstein.

Table 1 Patient characteristics and efficacy results of weekly irinotecan + docetaxel and non-pegylated liposomal doxorubicin +/- gemcitabine + G-CSF in the 27 patients with platinum-resistant or platinum-refractory ovarian cancer *n* (%)

	Weekly Irinotecan + docetaxel	Liposomal doxorubicin +/- gemcitabine + G-CSF
Included/planned	15/45	12/80
Years of study inclusion	2005-2006	2008-2011
Age (yr), median (range)	56 (33-77)	65 (46-77)
Previous regimens, median (range)	1.9 (1-4)	1.6 (1-3)
Platinum-refractory disease	8 (53)	1 (8)
Platinum-resistant disease	7 (47)	11 (92)
No. of previous chemotherapy regimens		
1	8 (53)	1 (8)
2	3 (13)	10 (83)
3	2 (13)	1 (8)
4	2 (13)	0 (0)
Previous taxanes	12 (80)	12 (100)
Complete response	1 (7)	0 (0)
Partial response	1 (7)	2 (17)
Stable disease	2 (13)	2 (17)
Progressive disease	11 (73)	8 (67)
Progression-free survival (mo), median (range)	2.8 (2.0-3.6)	2.1 (1.0-9.0)
Overall survival from study inclusion (mo), median (range)	10 (7.8-12.2)	10 (2.1-44.2)

Both studies were approved by the institutional Ethics Committees. For inclusion, patients had to sign an informed consent, to have no secondary cancer, no previous radiotherapy, no active infections, an adequate bone marrow function and liver parameters, as well as a serum creatinine of less than 2.5 × normal.

Progression was defined as the occurrence of new measurable disease by computed tomography scan and/or by chest X-ray.

In the first study, treatment consisted of 6 cycles of irinotecan at 55 mg/m^2 and docetaxel 25 mg/m^2, both administered on days 1, 8 and 15 of a 4 wk cycle.

In the second randomized phase Ⅱ study, either non-PEG L-DXR 60 mg/m^2 monotherapy on day 1 and PEG filgrastim on day 2 (arm A) or L-DXR at 50 mg/m^2 and GEM at 650 mg/m^2 on day 1 and GEM on day 8 (arm B) were administered every 4 wk, respectively. Patients in arm B received prophylactic filgrastim 5 μg/kg per day from days 3 to 6 and from days 9 to 12, respectively.

Pretreatment patient characteristics are shown in Table 1.

RESULTS

Treatment efficacy

Efficacy results of the two phase Ⅱ studies with irino-

Table 2 Worst grade 3 and 4 toxicities (National Cancer Institute-common toxicity criteria) per patient with platinum-resistant or platinum-refractory ovarian cancer undergoing weekly irinotecan + docetaxel and non-pegylated liposomal doxorubicin +/- gemcitabine + G-CSF *n* (%)

Grade 3 and 4 toxicity	Weekly irinotecan + docetaxel	Liposomal doxorubicin +/- gemcitabine + G-CSF
Patients	15 (100)	12 (100)
Neutropenia grade 4	0 (0)	2 (17)
Neutropenia grade 3	2 (13)	4 (33)
Febrile neutropenia grade 4	1 (7)	2 (17)
Leucopenia grade 3	2 (13)	3 (25)
Leucopenia grade 4	0 (0)	1 (8)
Thrombocytopenia grade 4	0 (0)	2 (17)
Nausea grade 3	0 (0)	1 (7)
Stomatitis/mucositis grade 3	0 (0)	3 (25)
Diarrhea grade 3	4 (27)	0 (0)
Diarrhea grade 4	1 (7)	0 (0)
Infection grade 3	0 (0)	2 (17)
Fatigue grade 3	1 (7)	2 (17)
Pain grade 3	1 (7)	2 (17)
Thromboembolism grade 3	2 (13)	1 (7)
Weight gain grade 3	1 (7)	0 (0)

Table 3 Serious adverse events observed in the two Austrian Arbeitsgemeinschaft für Gynäkologische Onkologie phase Ⅱ studies using irinotecan + docetaxel or liposomal doxorubicin +/- gemcitabine + G-CSF in patients with platinum-resistant and platinum-refractory ovarian cancer *n* (%)

Type of SAE	Weekly irinotecan + docetaxel (*n* = 15)	Liposomal doxorubicin +/- gemcitabine (*n* = 12)
Subileus and fatigue	0	3 (25)
Uncontrollable vomiting and fever	0	2 (17)
Febrile neutropenia	0	1 (8)
Pulmonary embolism/deep pelvic vein thrombosis	2 (13)	1 (8)
Pneumonia	0	1 (8)
Significant pleural effusion	0	1 (8)
Mucositis/diarrhea	1 (7)	1 (8)
Generalized edema	0	1 (8)
Total number of SAE	3 (20)	11 (91)

SAE: Serious adverse events.

tecan + docetaxel and L-DXR +/- GEM + G-CSF are shown in Table 1. In brief, the majority had platinum-resistant disease and the response rates were 14% and 17%, respectively. The median progression-free survival was less than 3 mo and the overall survival 10 mo, respectively.

Toxicity

Grade 3 and 4 toxicities are outlined in Table 2 and serious adverse events (SAE) in Table 3, respectively. Diarrhea was most prevalent in patients treated with irinotecan + docetaxel, while stomatitis/mucositis occurred in a quarter of patients treated with L-DXR +/- GEM, respectively. Table 3 shows the SAE observed in both studies. Following treatment with L-DXR +/- GEM, a total of 11 SAE occurred among the 12 patients included.

Both studies were stopped prematurely based on a decision of the AGO steering committee. It was felt that the harms for the patients in terms of toxicity were greater than its potential use.

Quality of life was examined in both phase Ⅱ studies. However, the early termination of both studies prohibited meaningful analyses.

Table 4 shows commonly used systemic therapy regimens in patients with platinum-resistant ovarian cancer.

DISCUSSION

Both phase Ⅱ AGO studies in patients with platinum-resistant ovarian cancer reported here exerted only a low therapeutic index. The rate of remissions was low and the toxicity significant. In the second phase Ⅱ study, G-CSF was prophylactically administered. However, its administration did not increase the tolerability of these regimens (Tables 2 and 3). Both studies were terminated prematurely.

In platinum-resistant or platinum-refractory ovarian cancer, treatment options are limited. Most patients die from the disease within 1 year[1,2] (Table 2). In this late stage disease, not only grade 3 or 4 toxicities but even the combination of several grade 2 toxicities, such as stomatitis, nausea, vomiting or fatigue, can deteriorate the patient´s performance status and motivation. Thus, the combination of cancer-related symptoms and toxicities is particularly critical when two-drug regimens are used.

There are only a limited number of studies published in platinum-resistant ovarian cancer, including one from our group in which combination therapy was found to be significantly effective on one hand and acceptably tolerated on the other[5]. Canfosfamide and PEG L-DXR showed a better therapeutic index than PEG L-DXR alone in one randomized phase Ⅱ study. Palmoplantar erythrodysesthesia and stomatitis were less pronounced in the combination arm[6].

Recently, single agent NKTR-102, a topoisomerase I-inhibitor polymer conjugate, demonstrated a response in 20% of patients after failure on PEG L-DXR[7].

In platinum-resistant disease, the induction of a remission is particularly necessary to reduce cancer-related symptoms, including subileus, abdominal distension and dyspnea[2].

Targeted therapies

In combination with tamoxifen, targeted therapy such as gefitinib, a tyrosin kinase inhibitor of the epidermal growth factor receptor, has shown only modest efficacy in platinum-resistant or platinum-refractory ovarian cancer. This regimen was associated with significant drug-related adverse events, including diarrhea and acne-like rash[8].

Bevacizumab is an antibody against vascular endo-

Table 4 Commonly used systemic therapy regimens in patients with platinum-resistant or platinum-refractory ovarian cancer

Chemotherapy regimen	Dose	Interval	Remarks
PEG-liposomal doxorubicin	40-45 mg/m^2 per day iv	day 1 every 4 wk	No alopecia, non-significant nausea/emesis, minor myelosuppression, significant hand-foot syndrome in 10% to 20%, respectively
Paclitaxel weekly	80 mg/m^2 per day iv (1-h-infusion)	Once weekly for three doses followed by 2 wk rest	Complete alopecia, no peroral premedication with dexamethasone necessary, minor myelosuppression, less neurotoxicity compared with 3-weekly paclitaxel
Gemicitabine	1000-1250 mg/m^2 per day iv	Days 1 + 8 every 3 wk	No alopecia, minor nausea/emesis, significant myelosuppression
Topotecan weekly	4 mg/m^2 per day iv	Days 1, 8, 15 every 4 wk	Moderate myelosuppression, rarely emesis/nausea, rarely significant alopecia
Bevacizumab	15 mg/kg per day iv	Day 1 every 3 wk	Significant hypertension, gastrointestinal perforations in up to 11%
Tamoxifen	20 mg/d	Daily	Response rate around 10%, mild toxicity

iv: Intravenously; PEG: Pegylated.

thelial growth factor. In a phase Ⅱ study, bevacizumab monotherapy resulted in a remarkable response rate in 16% in patients with platinum-resistant disease. However, gastrointestinal perforation occurred in as many as 11% of patients[9]. The prevalence of the latter complication was also high in a retrospective study which included heavily pretreated patients who had received a median of seven prior regimens. Bevacizumab was combined with cyclophosphamide, 5-fluoruracil, docetaxel or PEG L-DXR/GEM. The 35% response rate was encouraging[10].

In one phase Ⅱ study of bevacizumab and weekly topotecan, partial responses were seen in 24% and disease stabilization in another 36% of patients[11]. Although the response rate again was encouraging (46%), the combination of weekly PEG L-DXR and bevacizumab exerted significant toxicity, mainly including hand-foot syndrome and mucositis[12].

Thus, the ideal combination partner of bevacizumab remains to be defined. The Aurelia study is currently addressing this question (ClinicalTrials.gov identifier: NCT00976911).

Whether pertuzumab combined with GEM[13] or other regimens may expand the current armamentarium against platinum-resistant ovarian cancer needs confirmation in larger studies.

Antihormonal therapies

Antihormonal treatments such as tamoxifen or gonadotropin-releasing hormones have revealed remission rates in only about 10% of patients but, in addition, disease stabilizations in about one third of women treated[14,15]. Antihormonal effects do not seem to depend on the existence of hormone receptors of the tumor. One advantage of tamoxifen and similar agents is its mild toxicity.

In conclusion, a low therapeutic index was observed in both Austrian AGO phase Ⅱ studies using irinotecan + docetaxel and non-PEG doxorubicin +/- GEM + G-CSF, respectively. Both regimens cannot be recommended in platinum-resistant disease which remains a clinical dilemma. Bevacizumab seems to be active at this late-stage disease but may be associated with significant bowel toxicity. Studies defining combination partners for this monoclonal antibody are underway. Antihormonal therapy such as tamoxifen also has a place in the treatment of platinum-resistant and platinum-refractory ovarian cancer.

ACKNOWLEDGMENTS

The authors thank Dr. Johanna Ulmer, AGO Studienzentrale, for her support in this study. The contributions of the following members of the Austrian AGO study group are particularly acknowledged: Dr. Gerda Sallinger, Graz; Dr. Sabine Kreische, Kufstein; Dr. Beda Hartmann, Neunkirchen; Dr. Ute Pfleger and Dr. Monika Neuwirth, Wiener Neustadt; Dr. Petra Kohlberger, Vienna.

COMMENTS

Background

The development of platinum resistance in ovarian cancer is a major clinical problem. New effective chemotherapy regimens are urgently needed.

Research frontiers

In the situation of non platinum-sensitive recurrent ovarian cancer, patients suffer from significant tumor-related symptoms and chemotherapy is only moderately active. The median survival lies between 6 and 11 mo.

Innovations and breakthroughs

In the heavily pretreated population with platinum-resistant ovarian cancer, two phase Ⅱ studies were unable to establish a new combination regimen with a favorable therapeutic index.

Applications

None of the combination regimens investigated by the Austrian Arbeitsgemeinschaft für Gynäkologische Onkologie can be recommended.

Terminology

Platinum-refractory and platinum-resistant recurrence: Patients either have disease progression during first-line platinum-taxane therapy or recurrence within 6 mo following the last platinum-containing chemotherapy, respectively.

Peer review

This is an interesting article in this field. The randomized trial compares two treatment regimens which are different for two variables.

REFERENCES

1 **Markman M**, Webster K, Zanotti K, Peterson G, Kulp B, Belinson J. Survival following the documentation of platinum and taxane resistance in ovarian cancer: a single institution

experience involving multiple phase 2 clinical trials. *Gynecol Oncol* 2004; **93**: 699-701

2 **Doyle C**, Crump M, Pintilie M, Oza AM. Does palliative chemotherapy palliate? Evaluation of expectations, outcomes, and costs in women receiving chemotherapy for advanced ovarian cancer. *J Clin Oncol* 2001; **19**: 1266-1274

3 **Gordon AN**, Tonda M, Sun S, Rackoff W. Long-term survival advantage for women treated with pegylated liposomal doxorubicin compared with topotecan in a phase 3 randomized study of recurrent and refractory epithelial ovarian cancer. *Gynecol Oncol* 2004; **95**: 1-8

4 **Mutch DG**, Orlando M, Goss T, Teneriello MG, Gordon AN, McMeekin SD, Wang Y, Scribner DR, Marciniack M, Naumann RW, Secord AA. Randomized phase III trial of gemcitabine compared with pegylated liposomal doxorubicin in patients with platinum-resistant ovarian cancer. *J Clin Oncol* 2007; **25**: 2811-2818

5 **Petru E**, Angleitner-Boubenizek L, Reinthaller A, Deibl M, Zeimet AG, Volgger B, Stempfl A, Marth C. Combined PEG liposomal doxorubicin and gemcitabine are active and have acceptable toxicity in patients with platinum-refractory and -resistant ovarian cancer after previous platinum-taxane therapy: a phase II Austrian AGO study. *Gynecol Oncol* 2006; **102**: 226-229

6 **Vergote I**, Finkler NJ, Hall JB, Melnyk O, Edwards RP, Jones M, Keck JG, Meng L, Brown GL, Rankin EM, Burke JJ, Boccia RV, Runowicz CD, Rose PG. Randomized phase III study of canfosfamide in combination with pegylated liposomal doxorubicin compared with pegylated liposomal doxorubicin alone in platinum-resistant ovarian cancer. *Int J Gynecol Cancer* 2010; **20**: 772-780

7 **Garcia AA**, Vergote IB, Micha JP, Pippitt CH, Rao GG, Spitz DL, Reed N, Dark GG, Ibrahim EN, Armenio VA, Duska LR, Poole CJ, Gennigens C, Dirix LY, West J, Zhao C, Leung AC, Masuoka LK, Rustin GJS. The role of NKTR-102 in women with platinum resistant/refractory ovarian cancer and failure on pegylated liposomal doxorubicin (PLD). *J Clin Oncol* 2011; **29**: Abstr 5047

8 **Wagner U**, du Bois A, Pfisterer J, Huober J, Loibl S, Lück HJ, Sehouli J, Gropp M, Stähle A, Schmalfeldt B, Meier W, Jackisch C. Gefitinib in combination with tamoxifen in patients with ovarian cancer refractory or resistant to platinum-taxane based therapy--a phase II trial of the AGO Ovarian Cancer Study Group (AGO-OVAR 2.6). *Gynecol Oncol* 2007; **105**: 132-137

9 **Cannistra SA**, Matulonis UA, Penson RT, Hambleton J, Dupont J, Mackey H, Douglas J, Burger RA, Armstrong D, Wenham R, McGuire W. Phase II study of bevacizumab in patients with platinum-resistant ovarian cancer or peritoneal serous cancer. *J Clin Oncol* 2007; **25**: 5180-5186

10 **Wright JD**, Hagemann A, Rader JS, Viviano D, Gibb RK, Norris L, Mutch DG, Powell MA. Bevacizumab combination therapy in recurrent, platinum-refractory, epithelial ovarian carcinoma: A retrospective analysis. *Cancer* 2006; **107**: 83-89

11 **McGonigle KF**, Muntz HG, Vuky J, Paley PJ, Veljovic DS, Gray HJ, Malpass TW. Phase II prospective study of weekly topotecan and bevacizumab in platinum-resistant ovarian, fallo pian tube or primary peritoneal cancer. *Gynecol Oncol* 2009; **112**: S145

12 **Kouta H**, Kudoh K, Takano M, Kikuchi R, Kita T, Goto T, Furuya K, Kikuchi Y. The role of bevacizumab in combination with pegylated liposomal doxorubicin in patients with platinum-resistant recurrent or refractory ovarian cancers. *J Clin Oncol* 2011; **29**: Abstr 5018

13 **Makhija S**, Amler LC, Glenn D, Ueland FR, Gold MA, Dizon DS, Paton V, Lin CY, Januario T, Ng K, Strauss A, Kelsey S, Sliwkowski MX, Matulonis U. Clinical activity of gemcitabine plus pertuzumab in platinum-resistant ovarian cancer, fallopian tube cancer, or primary peritoneal cancer. *J Clin Oncol* 2010; **28**: 1215-1223

14 **Tropé C**, Marth C, Kaern J. Tamoxifen in the treatment of recurrent ovarian carcinoma. *Eur J Cancer* 2000; **36** Suppl 4: S59-S61

15 **Kristensen G**, Kaern J, Baekelandt M, Skeje-Jesensen T, de-Pont Christensen R, AvalI-Lundqvist E, Bergdahl M, Sanvei R, Hoegberg T, Grenmann S. Chemotherapy versus hormonal treatment in patients with platinum and taxane resistant ovarian cancer: A NSGO study. *J Clin Oncol* 2008; **26**: Abstr 5508

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World J Obstet Gynecol 2012 October 10; 1(3): I
ISSN 2218-6220 (online)

Acknowledgments to reviewers of *World Journal of Obstetrics and Gynecology*

We acknowledge our sincere thanks to our reviewers. Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of our World Series Journals. Both the editors of the journals and authors of the manuscripts submitted to the journals are grateful to the following reviewers for reviewing the articles (either published or rejected) over the past period of time.

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World J Obstet Gynecol 2012 October 10; 1(3): I
ISSN 2218-6220 (online)

Events Calendar 2012

January 26-30, 2012
55th All India Congress of Obstertrics and Gynaecology -2012
Banaras Hindu University, Varanasi, India

March 27-31, 2012
2012 American College of Medical Genetics Annual Clinical Genetics Meeting
Charlotte, NC, United States

May 3-5, 2012
Breast 2012 - IMPAKT
Brussels, Belgium

May 9-11, 2012
III Regional Conference of the Society of Obstetrics and Gynecology
Merida, Venezuela

May 9-12, 2012
EBCOG 2012 - 22nd European Congress of Obstetrics and Gynaecology
Tallinn, Estonia

May 9-12, 2012
19th European Congress on Obesity ECO2012
Lyon, France

May 10-12, 2012
ESSIC Annual Meeting
Porto, Portugal

May 17-19, 2012
Advances in Health Care for Women Over 40
Las Vegas, NV, United States

May 17-20, 2012
CPP 2012 - The 2nd International Meeting on Cardiac Problems in Pregnancy
Berlin, Germany

May 24-26, 2012
7th Brasilian Congress on Menopause and Climacteric
Sao Paulo, Brazil

June 5-8, 2012
10th RCOG International Scientific Congress (Royal College of Obstetricians and Gynaecologits)
Kuching, Sarawak, Malaysia

June 7-9, 2012
11th European Meeting Days of the French Society of Ginecology
Paris, France

June 13-16, 2012
XXIII European Congress of Perinatal Medicine
Paris, France

June 20-23, 2012
12th ESC Congress- Myths and misconceptions versus evidence on contraception
Athens, Greece

July 1-4, 2012
28th Annual Meeting of the European Society of Human Reproduction and Embriology
Istanbul, Turkey

July 5-6, 2012
British Gynaecological Cancer Society Annual Meeting
Londres, Cuba

July 7-14, 2012
33rd World Medical and Health Games - Antalya 2012
Antalya, Turkey

July 19-22, 2012
16th World Congress on Controversies in Obstetrics, Gynecology & Infertility (COGI)
Singapore, Singapore

August 5-8, 2012
Office Gynecology
Snowmass, CO, United States

August 6-10, 2012
XXXIX Ob Gyn national congress
Guatemala, Guatemala

September 5-8, 2012
21st Society of Laparoendoscopic Surgeon Annual Meeting and Endo Expo 2012
Boston, MA, United States

September 11-14, 2012
21st Annual Congress ESGE (European Society for Gynaecological Endoscopy)
Paris, France

September 19-21, 2012
3rd Congress of Neonatology and 1st Congress of Reproductive Medicine
Ciudad de Panama, Panama

September 20-22, 2012
11th Congress of the European Federation of Sexology
Madrid, Spain

September 24, 2012
Recent Updates in the Reproductive Endocrinology and Infertility International Symposium & Workshop
Riyadh, Saudi Arabia

October 3-6, 2012
23rd Annual Meeting the North American Menopase Society (NAMS)
Orlando, FL, United States

October 5-7, 2012
2012 International Conference on Stillbirth, SIDS/SUID and Infant Survival
Baltimore, Maryland

October 7-12, 2012
XX FIGO World Congress of Gynecology and Obstetrics
Rome, Italy

October 10-13, 2012
National Association of Nurse Practitioners in Women's Health 15th Annual Conference
Orlando, FL, United States

October 11-14, 2012
The 15th congress on Controversies in Obstetrics, Gynecology and Infertility (COGI) congress on Building Consensus in Gynecology, Infertility and Per
Barcelona, Spain

October 12-13, 2012
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October 13-16, 2012
IGCS 2012 - 14th Biennial Meeting of the International Gynecologic Cancer Society
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October 17-20, 2012
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October 17-20, 2012
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World J Obstet Gynecol 2012 October 10; 1(3): I-V
ISSN 2218-6220 (online)

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World Journal of Obstetrics and Gynecology

ISSN

ISSN 2218-6220 (online)

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World Journal of Obstetrics and Gynecology
Editorial Department: Room 903, Building D,
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No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjog@wjgnet.com
http://www.wjgnet.com
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Indexed and Abstracted in
Digital Object Identifier.

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Acknowledgments

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Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Write as mean ± SD or mean ± SE.

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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