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(SIGO)*



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Table of contents	73
Position paper on prevention of surgical site infections in obstetric and gynecological surgery	75
I. Cetin, F. Ciccarone, S. Danese, P. De Iaco, D. De Vita, M. Franchi, M. Guido, A. Mattei, E. Lomeo, G. Scambia, M. Perrone, P. Scollo	
Polycystic Ovary Syndrome in adolescents: an update	97
L. Caserta, A. Gregnuoli	
The adherence adequacy to antenatal care in alleviating the adverse maternal and neonatal outcomes of Iranian pregnant women: a retrospective-prospective study	107
M. Yousefi, L. Khedmat, N. Akbari, M. Kashanian, M. Moradi Lakeh	
In vitro fertilization and psychological stress: new insight about different routes of progesterone administration	119
D. Mele, F. Caprio, M.D. D'Eufemia, A. Schiattarella, D. Labriola, M.T. Schettino, N. Colacurci	
The role of serum potassium and sodium levels in the development of postpartum hemorrhage. A retrospective study	126
A. A. Privitera, M. Fiore, G. Valenti, S. Raniolo, A. Schiattarella, G. Riemma, G. Gullo, F. Sgalambro, S. Garofalo, S. D'Amico, M. G. Li Destri, V. Esposito, P. Murabito, C. Zangara, M. Fichera	
Hepatic mesenchymal hamartoma and placental mesenchymal dysplasia: an association ever less rare; a focus on current Knowledge	136
A. Cordisco, M. Di Tommaso, A.M. Buccoliero, R. Biagiotti	
Case report of prenatal diagnosis and surgical treatment of congenital ranula	141
E. Pappalardo, F.A. Gulino, C. Ettore, E. Bambili, E. Casella, S. Grimaldi, S. Cacciaguerra, G. Ettore	

MICRONUTRIZIONE HIGH IMPACT

BIODISPONIBILITÀ DEI NUTRIENTI PER LA SALUTE DELLA DONNA



Non tutto quello che assumiamo con la dieta e l'integrazione viene assorbito e metabolizzato dal nostro corpo e questo avviene per numerose ragioni:

- Fattori dipendenti dal soggetto (funzionalità della barriera intestinale, equilibrio del microbiota e delle mucose, celiachia, IBD).
- Fattori dipendenti dalle condizioni intestinali (produzione gastrica di acido cloridrico e pH, produzione pancreatica di enzimi, uso di IPP).
- Fattori dipendenti dalla forma chimica del nutriente (sale inorganico, sale organico, forme chelate, vitamine attive o pro-vitamine).

La biodisponibilità dipende anche dalla contemporanea assunzione di alimenti contenenti altri minerali o antinutrienti come i fitati che possono sequestrare i minerali rendendoli meno biodisponibili. Anche in un soggetto perfettamente sano alcune forme chimiche possono presentare seri problemi di assorbimento con conseguente aumento degli eventi avversi gastrointestinali.

L'assorbimento intestinale è molto basso per ossidi e idrossidi, basso per le forme inorganiche (cloruro, carbonato, solfato, pirofosfato), medio-alto per forme organiche (citrato, gluconato, picolinato, glicerofosfato), alto per forme chelate (chelati con amminoacidi come i bisglicinati).

Gran parte degli eventi avversi gastrointestinali dovuti all'assunzione di ferro per os potrebbero essere evitati fornendo forme ad alta biodisponibilità, come il **bisglicinato di ferro**. Lo stesso dicasi per il **magnesio**, causa frequente di diarrea e dolori addominali se fornito in forma inorganica ma invece perfettamente tollerato in forma di **glicerofosfato** o bisglicinato.

Carenze subcliniche di magnesio e ferro sono molto frequenti nella popolazione femminile.

Uno studio europeo^{1,2} ha stimato che oltre l'80% delle donne non apporta quantitativi sufficienti di magnesio con la dieta e questo si correla a crampi, clonie, ipereccitabilità neuromuscolare, maggiore suscettibilità allo stress.

La biodisponibilità delle vitamine dipende in misura maggiore da quello che succede dopo l'assorbimento, ovvero dalle **trasformazioni necessarie per renderle utilizzabili dall'organismo**.

Per esempio i folati e la cobalamina devono essere metilati per entrare nei processi biologici per cui sono indispensabili. **Fornire forme già metilate semplifica il lavoro dell'organismo**.

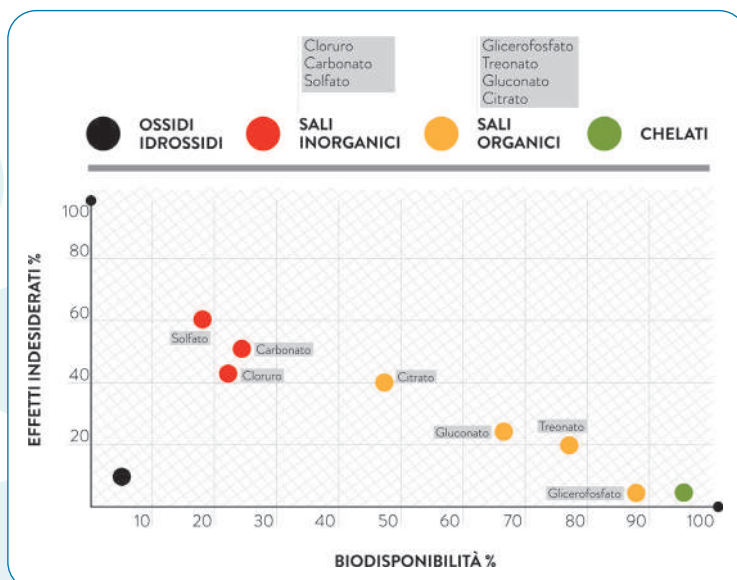
Non dimentichiamo che in Italia oltre il 20% della popolazione presenta un polimorfismo limitante dell'enzima MTHFR, deputato all'attivazione dei folati (provenienti dagli alimenti o da acido folico). Nei soggetti con polimorfismi genetici limitanti l'efficienza dell'enzima può essere ridotta fino al 65% con potenziali rischi di iperomocisteinemia e di difetti fetali del tubo neurale nel caso di una gravidanza. Anche in questo caso esiste una soluzione semplice: **fornire al posto dell'acido folico una forma attiva di folato, come il 5 metil-tetraidrofolato, e forme attive di B12 come la metilcobalamina**. Le forme attive delle vitamine B sono facilmente accessibili e rappresentano ad oggi l'unica soluzione per contrastare i potenziali rischi dei difetti di metilazione.

Le vitamine liposolubili (A,D,K,E) vengono assorbite nell'intestino tenue a livello del digiuno e il loro assorbimento e la loro biodisponibilità sono facilitati da una contemporanea assunzione di grassi.

La biodisponibilità dei micronutrienti rappresenta un fattore critico nel successo dei trattamenti di integrazione alimentare e dovrebbe essere considerato con maggiore attenzione dai professionisti della salute al fine ottenere interventi più efficaci e ben tollerati in ogni tipologia di pazienti.

¹ López-Sobaler et al. Adequacy of Usual Vitamin and Mineral Intake in Spanish Children and Adolescents: ENALIA Study. *Nutrients*. 2017 Feb 13;9(2).

² Olza et al. Reported Dietary Intake, Disparity between the Reported Consumption and the Level Needed for Adequacy and Food Sources of Calcium, Phosphorus, Magnesium and Vitamin D in the Spanish Population: Findings from the ANIBES Study. *Nutrients*. 2017 Feb 21;9(2).





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Position paper on prevention of surgical site infections in obstetric and gynecological surgery

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ABSTRACT

Surgical site infections represent about a quarter of all infections that develop in the hospital setting.

Far from being solved, this problem is expected even increasing in the next years, mainly for the increase in prevalence of obesity and diabetes and the increasing spread of antibiotic resistance. Obstetrics and Gynecology represent, for frequency of interventions, incidence and impact, one of the major fields of interest.

Caesarean section is one of the most frequent surgical procedures in women and is the one most likely to get complicated with surgical site infection. According to some estimates, up to 12% of cesarean sections will be complicated by surgical site infection with repercussions on women and newborn health, and high costs for the community.

With regard to Gynecology in particular, Gynecological Oncology often requires extensive interventions with a high risk of infections.

The Italian Society of Gynecology and Obstetrics (SIGO) has collected a group of colleagues, from all over Italy and from different professional areas, to "update" and "adapt" the most important international guidelines and recommendations on the prevention of surgical site infections in the Italian clinical practice of obstetric and gynecological procedures, in compliance with current legislation.

The aim of the document is to inform healthcare personnel to strictly adopt standardized procedures and to use for antiseptics pharmaceutical specialties with specific indications.

SOMMARIO

Le infezioni del sito chirurgico rappresentano circa un quarto di tutti le infezioni che si sviluppano nell'ambiente ospedaliero. Lungi dall'essere risolto, questo problema è sembrerebbe in crescita nei prossimi anni, soprattutto per l'aumento della prevalenza di obesità e diabete e la crescente diffusione della resistenza agli antibiotici.

Ostetricia e ginecologia rappresentano, per frequenza degli interventi, incidenza e impatto, uno dei principali campi di interesse. Il parto cesareo è una delle più frequenti procedure chirurgiche nelle donne ed è quello che ha più probabilità di ottenere infezioni chirurgiche del sito. Secondo alcune stime, fino al 12% dei parti cesarei sarà complicato da un'infezione del sito chirurgico con ripercussioni sulle donne, sulla salute dei neonati e costi elevati per la comu-

nità. Per quanto riguarda la ginecologia in particolare, l'oncologia ginecologica richiede spesso interventi estesi con un alto rischio di infezioni.

La Società Italiana di Ginecologia e Ostetricia (SIGO) ha raccolto un gruppo di colleghi, provenienti da tutta Italia da diverse aree professionali, per "aggiornare" e "adattare" le più importanti linee guida e raccomandazioni internazionali sulla prevenzione delle infezioni da siti chirurgici nella pratica clinica italiana delle procedure ostetriche e ginecologiche, nel rispetto della legislazione vigente.

Lo scopo del documento è quello di informare il personale sanitario a adottare rigorosamente procedure standardizzate e ad utilizzare per le specialità farmaceutiche antisepsi con specifiche indicazioni.

INTRODUCTION

Technological innovations always move the frontiers forward in obstetric and gynecological surgery, offering today to women and to products of conception, opportunities once unthinkable.

However, this must not let us forget some "historical", fundamental, topics of our discipline that never cease to be crucial, for which we cannot let our guard down and a constant updating on preventive and therapeutic methods and possibilities is necessary.

The field of surgical infections owes the birth to an obstetric colleague, the Hungarian Ignac Semmelweis, in the mid-1800s, he had the intuition of the infection transmission to one subject to another and the additional illumination that often is the health professional who transmits the infection.

Even today in the Obstetrics and Gynecology departments, despite significant progress in prevention and control, surgical site infections (SSI) represent a problem with an important impact on individual, economic and social terms. Obstetrics and Gynecology represent, for frequency of interventions, incidence and impact of infections, one of the major fields of interest. Caesarean section is one of the most frequent surgical procedures in women and is the one most likely to get complicate with surgical site infection. According to some estimates, up to 12% of cesarean sections will be complicated by surgical site infection (from cutaneous infection up to endometritis or abscess) with repercussions on women and newborn health, and high costs for the community.

With regard to Gynecology in particular, Gynecological Oncology often requires extensive interventions with a high risk of infections.

In the prevention of surgical site infections, healthcare personnel must strictly adopt standardized procedures and pharmaceutical specialties with specific indications must be used for antisepsis.

In consideration of all these aspects, the Italian Society of Gynecology and Obstetrics (SIGO) has brought together a group of colleagues, from all over Italy and different professional areas, to "update" and "adapt" the most important international guidelines and recommendations on

the prevention of surgical site infections in the Italian clinical practice of obstetric and gynecological procedures, in compliance with current legislation and finally disseminate them to all SIGO members.

Special thanks to Nicola Petrosillo, the Director of the Clinical and Research Department in Infectious Diseases, National Institute for Infectious Diseases "L. Spallanzani", IRCCS - Rome for the support provided.

SSI DEFINITION AND EPIDEMIOLOGY

According to the definition of the ECDC (European Center for Disease Prevention and Control), postoperative infection that occurs within 30 days of a surgical procedure, or within one year in case of implantation of permanent device, is defined as a SSI (1).

The World Health Organization has defined the reduction of the risk of SSI as one of the 10 priority objectives for safe surgery (2).

Surgical site infections represent about a quarter of all infections that develop in the hospital setting (so-called nosocomial infections) (3).

On the basis of this definition of SSI, it can be seen that the measurements based only on hospital surveillance considerably underestimate the frequency.

SSI can be classified (4) into:

- incisional (about 2/3);
 - superficial when affects skin and subcutaneous tissue;
 - deep when reaches the fascia or muscle plane;
- organ and space infections (about 1/3) when affect any other anatomical site involved in the surgery.

There are numerous factors influencing the probability of incidence of SSIs. Surgical intervention can be classified by the probability and degree of contamination of the wound at the end of surgery. One of the most used classifications is the following (5):

Class I / clean	Surgical interventions on non-infected wound, without affecting the respiratory, gastrointestinal, genitourinary tract. Interventions closed at first instance and, when necessary, drained with closed drains. Surgical procedures consecutive to non-penetrating trauma must be included in this category if they meet the previous criteria.
Class II/ clean contaminated	Surgical interventions affecting the respiratory, gastrointestinal or genitourinary tract, in controlled conditions and without significant contamination of the wound. In particular, surgical interventions on the biliary tract, appendix, vagina and oropharynx are included in this category, provided that there is no evidence of infection and no interruption of aseptic techniques
Class III / contaminated	Surgical interventions consecutive to a recent, open trauma. Interventions in which asepsis is not guaranteed (e.g. open heart massage) or there is significant spreading of the gastrointestinal contents or surgical interventions involving an acute, non-purulent inflammatory process.
Class IV / dirty-infected	Interventions on long-standing traumas with tissue retention and interventions involving acute purulent infectious processes or in the presence of viscera perforation. In these procedures, the microorganisms causing the postoperative infection are present in the operating field before the procedure.

In addition, the risk is also dependent by the conditions of patients who are classified into 5 groups according to life expectancy defined by the American Society of Anesthesiology (6):

ASA 1	Healthy patients undergoing surgery for a localized pathology.
ASA 2	Mild or moderate systemic disease (well-controlled high blood pressure, history of asthma, anemia, smoking, well-controlled diabetes mellitus, mild obesity, age <1 year or > 70 years, pregnancy).
ASA 3	Serious systemic disease (angina, myocardial infarction, uncontrolled hypertension, symptomatic respiratory disease, severe obesity).

ASA 4	Severe life-threatening systemic disease (unstable angina, heart failure, liver or kidney failure).
ASA 5	Dying patient with low survival expectations.

The National Nosocomial Infections Surveillance (NNIS) index allows to divide patients into four categories (0, 1, 2, 3) according to their risk of developing a SSI. This index allows to calculate the infection rates taking into account certain risks related to patients and interventions, in comparing different hospitals.

The index is based on three parameters to which the value 0 or 1 are assigned.

	0	1
Contamination class	<III	≥ III
Score ASA	<3	≥ 3
Duration of the intervention	The duration is below the 75th percentile of the surgical intervention	The duration is above the 75th percentile of the surgical intervention

The 75th percentile of the duration of the intervention is therefore a stable value (7). The type of intervention has a significant influence (8). Looking at European surveillance studies, a study conducted by ECDC in 16 countries based on post-discharge surveillance showed the highest overall SSI rate in Europe 30 days after colorectal surgery (9.5 episodes per 100 operations), followed by cardiac surgery (3.5% in cesarean operations) (1).

Two national SSI surveillance studies have been carried out in our country. The first study (9) was conducted in 48 Italian surgeries, studying cases up to 30 days after surgery. The overall incidence rate of SSI was 5.2% with a maximum of 18.9% for colorectal surgery. At a multivariate analysis, factors independently associated with SSI risk were: emergency intervention (OR 1.73 with 95% CI 1.22- 2.44; $p = 0.02$), NNIS score higher than 0 (OR 3.34 with 95% CI 1.41-7.93; $p = 0.006$), preoperative hospital stay longer than 1 day (OR 1.45 with 95% CI 1.06-1.98; $p = 0.02$) and use of drainage (OR 2.17 with 95% CI 1.39-3.43; $p < 0.001$).

Data from the national SSI surveillance program during the period 2009 to 2011 show (10) an incidence of 2.6%, which in one third of the cases was represented by a deep or involving organ/

space infection. At a multivariate analysis the duration of the intervention above the 75th percentile (OR 1.52 with 95% CI 1.32-1.74; $p < 0.001$), an ASA score higher than or equal to 3 (OR 1.42 with 95% CI 1.22-1.65; $p < 0.001$), a preoperative hospital stay longer than or equal to 2 days (OR 1.22 with 95% CI 1.05-1.41; $p < 0.05$), and an emergency intervention (OR 1.29 with 95% CI 1.11-1.51; $p < 0.05$) were associated with a higher risk of SSI, while a laparoscopic procedure was associated with a significant lower risk of SSI (OR: 0.49 with 95% CI 0.40-0.61; $p < 0.001$). It should be noted that around 50% of SSIs was identified ten days after surgery, when 90% of the patients had already been discharged. From an epidemiological projection point of view, the aging of population, the increase in prevalence of obesity and diabetes and the increasing spread of antibiotic resistance is likely to increase SSI rate in the coming years.

With regard to the epidemiology of interventions in the obstetric-gynecological area, a subject of particular interest is the caesarean section (CS), considering the high number of CS, the particular immunological condition of pregnant women and the possibility this intervention could have urgent characteristics. The Multi-country Survey on Maternal and Newborn Health made by the World Health Organization in 29 countries showed a total percentage of caesarean sections equal to 28.6% and an antibiotic prophylactic coverage of 87.3% (11).

The risk of post-partum infection after caesarean delivery is approximately 5 times higher than after vaginal delivery (12). SSI frequency after caesarean sections varies in literature from 5 to 12% (13)(14)(15) and can vary from skin infection up to endometritis or abscess around the hystero-rafia (16).

In ECDC surveillance, the average incidence of SSI 30 days after caesarean delivery was 2.9%; there are large differences between countries in the incidence of SSI, the highest cumulative incidences are reported in countries with intensive post-discharge surveillance system (Norway, United Kingdom). 87% of the total is represented by superficial SSI, 10% by deep SSI and 3% by SSI involving organ/space. 16% of SSI referred to diagnoses made in hospital, while 84% were post-discharge. Characteristics of women who developed SSI after caesarean were the following:

Age (median)	31 years
Surgery duration (median)	40 minutes
Duration of post-intervention stay (median)	5.6 days
Emergency surgery	53%
Antibiotic prophylaxis	89.1%

According to ECDC data in Italy the recorded incidence is 1.8% (1).

One of the factors most frequently associated with infectious complications after cesarean delivery is prolonged labor (more than 6-8 hours) after rupture of the membranes. In fact with the rupture of the membranes disappears the protective barrier of the uterus against bacterial infections (17). Additional risk factors are systemic diseases, poor personal hygiene, obesity and anemia (17) (18).

After cesarean delivery, the most frequent surgery in the obstetric-gynecological area is hysterectomy mainly due to fibroids, prolapses, hyperplasias, endometriosis and cancer.

The extent of the surgery and the surgical approach can be different.

The rate of SSI after abdominal hysterectomy varies in a range between 1% and 4% with a higher risk when compared to the vaginal approach (19). Laparoscopic hysterectomy showed a significantly lower average rate of infections compared to the laparotomic technique (1.15% vs 3.44%) (20).

In oncological surgery, women can have an immunosuppression state due to therapies or to the psychological status; furthermore, previous radiant therapies may represent a local risk factor for SSI (21).

SSI BURDEN

The onset of nosocomial infections has an important effect on patients and their families due to the increase of mortality and morbidity rate and it has also direct and indirect costs.

Direct costs concern:

- hospital and post-discharge care (hospitalization, intensive care unit, instrumental examinations, drugs, new surgery, rehabilitation therapy, outpatient medical checks, home care);
- NON-healthcare assistance provided by

family members outside of working hours for assistance, treatment and medical visits.

Indirect costs concern:

- possible loss of productivity of the patient and the caregiver;
- the so-called intangible costs i.e. mental, physical and social suffering and negative implications on quality of life.

In Europe, are estimated at 4.1 million cases of hospital infections, with an estimated annual economic load of around 7 billion euros, only for direct costs (8).

In Italy, it has been hypothesized that hospital infections generate approximately 1 billion euros of additional healthcare costs, mainly due to the increased hospital stay (up to € 28,000 for a patient in Intensive Care) (22).

SSI represents the most frequent hospital infections, but its economic impact is extremely variable in consideration of the type of intervention, and of the different site that may be affected, the type and severity of infection, but also of the differences in the methods of management and prevention of infections in different countries. A patient with SSI usually extends his hospital stay by 7-10 days, with an increased risk of staying in an intensive care unit (+ 60%), of new hospitalizations (up to 5 times) or even exitus (up to doubles) (23,24,25,26). Infections occurring after discharge carry the risk of new hospitalizations (27,28). Unfortunately, SSI is more and more frequently caused by antibiotic-resistant microorganisms that make harder the recovery, with the risk of more severe sequelae, even more extended hospitalization and increased costs (29). Long-term consequences of SSI can be persistent pain, unaesthetic scars, joint limitation, impact on quality of life (30,31,3).

Focusing on European data, in the United Kingdom 14,300 SSI were analyzed over about two years, for which an average hospital stay of 10 days was calculated (95% confidence interval: 7-13 days), which is approximately twice compared to patients without SSI (32).

In Germany the estimated additional cost due to SSI varies between 7,500 and 16,000 euros (33). An aspect often underestimated, especially in the past, is the impact on patient's quality of life. In SSI patients the values of Health-Related

Quality of Life (HRQoL) remain low for months or even years compared to subjects without infection(34) (35).

A final aspect of great relevance concerns the cost relating to legal disputes that patients increasingly start against the hospital.

Surgical site infection in obstetric patients carries implications not only for patients and for the society, but also for the newborn, because a complicated puerperium can interfere in the mother-newborn relationship, making hard the breastfeeding and the creation of a real bond between them (36).

MAIN GUIDELINES AND RECOMMENDATIONS FOR PREVENTION OF SSI SPECIFICALLY IN THE OBSTETRIC-GYNECOLOGICAL FIELD

Fortunately, a good percentage of SSI, up to 65%, can be prevented (37).

The prevention concerns health professionals and health procedures, the environment and materials in which the procedures take place, but also patients who must be considered as the gateway for the pathogens responsible for many SSI (38,39,40).

Table I schematically shows the main risk factors for SSI.

Table I. Risk Factor (10,41).

RELATED TO PATIENTS	RELATED TO THE TYPE OF SURGERY	RELATED TO OPERATOR
Age	Site Contaminated	Suitability of operating procedures
Comorbidities	Urgency Laparotomy/laparoscopy	
ASA score ≥ 3		
Preoperative hospital stay at least 2 days		

Active SSI surveillance is inherently associated with lower incidence rates, on the contrary intermittent interruptions of an active surveillance have shown a new increase in SSI rates (42). An interesting surveillance program carried out in Italy (10) has shown that a plan for detection and

reporting of hospital infections, in hospitals participating for more than 2 years, was able to lead to a reduction of 29 % of SSI rates.

In Italy there is a particular focus on quality and safety in health services through programming policies and service management and the practice of the Integrated Clinical Government (43,44).

Adverse events related to care processes are unexpected events resulting in unintentional and undesirable damage to patients, they can be divided between preventable and non-preventable; an adverse event attributable to a medical error is a preventable adverse event. SSI is a care-related infection and therefore can be assimilated to a preventable adverse event.

According to the Integrated Clinical Government, risk management concerns not only to clinical activities, but also technological-environmental, organizational aspects without forgetting the care appropriateness and sustainability.

The Gelli-Bianco law decree n. 24 March 8,2017, promotes the adoption of guidelines and good practices based on evidences (45). From a practical point of view, all health professionals are involved in patients taking care and sanitary facilities are called to actively engage monitoring strategies, prevention and risk management and to promote the implementation of specific protocols to improve guidelines.

In approaching the recommendations, it should be considered that evidence-based surgery, behavior and/or practice, if adequately carried out, will improve the quality and outcomes of procedures with a better effect than would be obtained if implemented separately (46).

The application of recommendations concerns all healthcare personnel and facility as a whole: surgeons, nurses, instrumentalists, operating room technicians, anesthesiologists and any other healthcare professional involved in the pre, intra and post-operative management of the patient, not excluded - in certain aspects - other crucial personnel such as infectious disease specialists, pharmacists, sterilization staff, up to decisional and organizational hospital staff (health department, quality and risk management staff, surgical, anesthesia and resuscitation department directors, nursing and pharmacy and organization and management of operating room

managers) in order to convey the importance and the cost-effectiveness standardization for patient safety.

In this document, among all evidence-based materials and recommendations included in the international guidelines for SSI prevention, are taken in consideration those fundamental in terms of strength of scientific evidence and describing perioperative procedures concerning operator habits and facility organization:

- global guidelines on SSI prevention, made by the World Health Organization (WHO). First published on November 3rd, 2016. Structured into 29 recommendations, the quality of evidence currently available (“very low”, “low”, “moderate”, “high”) is provided for each recommendation, but also the strength of the recommendation itself.
<http://www.who.int/gpsc/ssi-guidelines/en/>
- Centers for Disease Control and Prevention (CDC), published on May 3rd, 2017 on the surgical section of JAMA.
<https://www.cdc.gov/infectioncontrol/guidelines/ssi/index.html>
- Italian Consensus Document “Recommendations for perioperative prevention of surgical site infections” in which, referring to the most important international Guidelines published by WHO and CDC, are analytically examined the evidence-based recommendations that authors identify as crucial for SSI perioperative prevention based on the consistency of scientific evidences supporting them, the new data, the cost-effectiveness tests and the strength of recommendations.

PATIENT PREPARATION AND ANTIBIOTIC PROPHYLAXIS

Nutritional Support

Considering oral or enteral administration of nutritional supplements in order to prevent the onset of SSI in malnourished patients undergoing major surgery (WHO 2016, conditioned/very low).

According to a meta-analysis conducted by the

LG WHO 2016 working group, in major oncological surgery, malnourished patients (body mass index <18.5 and/or body weight 15-20% lower than the normal for age and height) fed with multiple nutritional preparations, showed a preventive effect towards SSI compared to a standard nutritional support.

Preoperative shower

Patients should shower or bathe, including hair washing, the previous day or the day of surgery, using normal soap or an antiseptic soap.

(WHO 2016, conditioned/moderate; CDC 2017, IB). It is not known what is the optimal time to have a preoperative shower or bath, the total number of soap or antiseptic applications and the effectiveness of cloths soaked in chlorhexidine in order to prevent SSI.

(CDC 2017, no recommendation/unresolved topic).

The preoperative bath or shower all over the body is considered a good clinical practice to reduce the bacterial load, especially at the incision site, remembering that the first source of infection is represented by microorganisms present on the skin.

From a practical point of view, the patient can have the preoperative shower at home or in the hospital bathroom if she is hospitalized, although if she is bedridden the ward staff must wash the patient.

The practice of the preoperative shower can be adequately explained and its importance must be underlined before surgery and reported in the instruction sheet given to the patient before hospitalization. At the time of admission, the execution and adequacy of the practice must be verified. Particular attention for laparoscopic surgery must be paid to navel hygiene.

Trichotomy

Routine preoperative hair removal should be avoided; trichotomy must be performed only when necessary, i.e. in case hair, at or around the surgical site, interferes with the surgery. (WHO 2016, strong / moderate; CDC 2017 reaffirms CDC recommendation 1999).

- When necessary, it must be carried out,

exclusively with an electric clipper, the day of surgery (WHO 2016, strong/moderate; CDC 2017 reaffirms CDC recommendation 1999).

- It is believed that the safest time to have trichotomy is immediately before surgery (WHO 2016; CDC 2017 reaffirms CDC recommendation 1999).
- It is not possible to recommend or not the use of depilatory creams as the indications are controversial (WHO 2016).
- The razor trichotomy with traditional blades is absolutely not recommended both in the preoperative phase and in the surgery room (WHO 2016, strong/moderate).

This recommendation undermines the traditional surgical preoperative preparation based on "fasting, enema and trichotomy".

Having a completely hairless surgical field was considered protective against SSI, but today, on the contrary, trichotomy is considered a risk factor for SSI as it can cause skin microtraumas and abrasions that favor bacterial colonization of the surgical site in particular if carried out with a traditional razor.

In cases where the surgical field is in an area where hairs must be removed because they interfere with the surgical activity, the traditional razor must not be used, an electric clipper which cuts the hair at the base, 2-3 mm from the skin is indicated. The electric clipper must have disposable or reusable razor blades, which can be properly disinfected for every new patient, taking attention in the staff training regarding supply, use and maintenance of the clippers.

The time aspect should not be underestimated because, when absolutely necessary, the trichotomy must be performed maximum two hours before surgery; therefore from a practical point of view, at the time of the call or even in the surgery preparation room (41).

Women must be informed not to shave independently before admission and this information must be reported in the information booklet.

As for hair removal by wax, there is no evidence, but certainly due to the risk of micro-

injuries it must be done at least 10 days before surgery.

PERIOPERATIVE ANTIBIOTIC PROPHYLAXIS (PAP)

- PAP should be administered only when indicated according to the type of surgery (WHO 2016, strong/low; CDC 2017, IB);
- It must be made within 120 minutes from the incision, taking into account the half-life of the antibiotic (WHO 2016, strong/moderate), that is, in a timing that allows to reach an effective concentration in the serum and tissues at the time of incision (WHO 2016; CDC 2017, IB);
- In cesarean section it is preferable to administer PAP before the skin incision (CDC 2017, IA);
- It is recommended not to continue PAP after suturing the surgical incision (WHO 2016, strong/moderate), even in presence of drainage (WHO 2016, conditioned/low; CDC 2017, IA).

When considering the opportunity of a Perioperative Antibiotic Prophylaxis (PAP) to prevent SSI, it should be considered the assessment of the risk of side effects, the increase of antibiotic resistance and infections by *C. Difficilis* (47). The effectiveness of PAP is always closely linked to the kinetics of the antibiotic used: the shorter the half-life of the antibiotic used, the closer must be the administration at the time of incision of the skin. The antibiotic characteristics must ensure sufficient blood and tissue concentration for the entire duration of the surgery, because insufficient concentrations at the time of suturing have even proven to favor the development of SSI. It is reasonable to administer an additional intraoperative dose of antibiotic in case of surgeries lasting longer than twice the half-life of the antibiotic or in case of significant blood loss during surgery (> 1500 ml in the adult). It is underlined the absence of indications to continue the administration of antibiotic in absence of signs of infection after the end of surgery.

The obstetric population represents a particular challenge for antibiotic prophylaxis, because the transplacental passage of antibiotic to the fetus

must be considered. In the past, the administration of antibiotic prophylaxis was delayed until after cord clamping to avoid the passage of antibiotics to the fetus. The fears related to the administration of antibiotic to the newborn concerned the possible masking of a neonatal infection, the interference with the diagnostic-therapeutic path of a possible sepsis, and the selection of antibiotic resistant bacterial strains that can affect the newborn (46). Based on these theoretical risks, the Centers for Disease Control and Prevention's Guideline for Prevention of Surgical Site Infection, in 1999 established, with a high level of evidence, that for high-risk cesareans, antimicrobial agents should be administered immediately after cord clamping rather than preoperatively (4). A meta-analysis comparing the administration of antibiotic prophylaxis before skin incision versus antibiotic prophylaxis after cord clamping concluded that antibiotic prophylaxis before incision, in caesarean sections, not only decreased the incidence of postpartum endomyometritis and the overall incidence of infectious episodes, but also did not adversely affect any neonatal parameters (47). Several studies have been carried out to evaluate the impact of the timing of antibiotic prophylaxis on postoperative infectious complications and there was evidence of a decrease in infectious complications with antibiotic administration before skin incision compared to post-clamping administration (48,36).

In 2010 a Cochrane review concluded that the antibiotic prophylaxis compared to the absence of prophylaxis was associated with a reduction in feverish episodes, wound infections, endometritis and other mother's serious infectious complications, but data were insufficient to compare the timing of antibiotic administration (49).

The American College of Obstetricians and Gynecologists (ACOG), in accordance with the recommendations of the National Surgical Infection Prevention Project (50) recommended antibiotic prophylaxis for all caesarean sections and concluded that the administration should be performed within 60 minutes from start (51).

A Cochrane systematic review, that analyzed 10 trials and a total of 5041 women, finally showed that PAP is more effective if administered before incision, rather than after clamping the umbilical cord, in reducing maternal infectious complications. In particular, women who received

antibiotics pre-operatively were 46% less likely to develop endomyometritis and 41% less likely to develop surgical wounds than women who received the antibiotic after umbilical cord clamping (52).

Bactericidal levels against group B streptococcus were obtained in maternal, fetal and amniotic fluid samples even 5 minutes before ampicillin administration (54). Data are in harmony with the results of the Cochrane review aimed at defining which antibiotics were most effective in reducing the incidence of infectious complications in cesarized women (55). The most commonly used antibiotic in elective cesarean delivery is cefazoline: pharmacokinetic studies show that the MIC (minimum inhibitory concentrations) for group B streptococcus are reached in maternal, fetal and amniotic fluid samples within 30 minutes after administration (53). In non-elective cesarean delivery, the choice is directed towards a second generation cephalosporin, an ureidopenicillin or an aminopenicillin and betalactamase inhibitor association (47). In gynecological surgery, the PAP administration resulted in a reduction of the infection rate and hospital costs (56). Antibiotic prophylaxis has always proven to be significantly effective in SSI prevention, both in abdominal (57) and vaginal hysterectomies (58). As far as laparoscopic gynecological procedures are concerned, there are some evidence that antibiotic prophylaxis seems unnecessary (59), but current recommendations do not distinguish between laparoscopic or laparotomic approach regarding the need of PAP (60).

ORAL ANTIBIOTIC PROPHYLAXIS

The administration of preoperative oral antibiotics in combination with mechanical bowel preparation (MBP) is suggested to reduce the risk of SSI in adult patients, candidates for colorectal elective surgery (WHO 2016, conditioned/moderate):

- MBP alone (without oral antibiotic administration) should not be used for reducing SSI in adult patients, candidate for colorectal surgery (WHO 2016, strong/moderate).

Oral administration of antibiotics in combination with preoperative administration of glycol-polyethylene or sodium phosphate solutions to induce bowel emptying (MBP) showed to reduce significantly the rate of SSI compared to MBP alone, therefore, in case of mechanical preparation, oral antibiotic prophylaxis should also be administered, both in addition to intravenous antibiotic prophylaxis, when appropriate.

Obstetric-gynecological surgery must be considered mostly "normal" abdominal surgery, fast track, without the need for abdominal preparation and oral antibiotic prophylaxis, but in Gynecological Oncology interventions involving the rectum, prophylaxis must be applied.

ANTISEPSIS

Skin preparation of the surgical team

Before entering the surgery room, wash your hands with non-medicated soap to remove organic material and reduce the bacterial load of the skin (for the correct hand hygiene in health sector, refer to the specific gl on the subject):

- before putting on sterile gloves, perform the surgical preparation of the hands and forearms of health personnel by washing with antiseptic soap or by applying hydro-alcoholic gel.

(WHO 2016, strong/moderate).

One of the cornerstones in SSI prevention is the surgical preparation of hands to keep the contamination of the surgical field at low levels, reducing the entry of microorganisms coming from the surgeon's skin into the surgical incision, especially in case of breakage of sterile gloves during surgery.

After removing all kind of jewelries and, if present, the nail polish (48), antiseptic soaps should be used, according to the manufacturer's instructions, generally for 2-5 minutes.

If a hydro-alcoholic gel is used, a product with a long-lasting action (for example chlorhexidine based) according to the manufacturer's instructions should be preferred, remembering that the effectiveness of the alcohol-based gel can be reduced when the product is applied on a not perfectly dry skin.

Sterile wipes and towels must be available for drying.

PATIENT SKIN ANTISEPSIS

Before starting the surgical site antiseptics, cleanse the skin thoroughly around the incision area to remove coarse contamination (CDC 2017):

- patient's skin antiseptics must be performed with an alcoholic antiseptic solution based on chlorhexidine gluconate. In people allergic to chlorhexidine, the antiseptics with alcoholic iodopovidone represents a second choice, if applied correctly and, possibly, in a 10% alcoholic solution.
- The use of colored products is recommended as an opportunity to verify the correct application of the product.
- The use of the disposable applicator, compared to the traditional method using gauze and multipurpose containers, improves the safety (risk of fire and contamination), standardization (correct dose of antiseptic) and practicality (time of application) of the procedure.
- There is currently no evidence in favor of repeating the antiseptics before the closure of the surgical incision.

(CDC 2017, no recommendation/unresolved topic).

Up to 10⁶ bacteria per cm² are estimated to be present on human skin, 80% of the bacteria are found in the first five layers of the epidermis, while the remaining 20% are closely related to the skin annexes (sebaceous glands, sweat glands and hair formations) (36).

Any action that alters the integrity of the skin (from a puncture of a vascular access to a surgical incision), decreases the barrier function towards infections and allows the microorganisms present to reach the circulatory stream or the tissues.

In the SSI case, patient's endogenous skin microflora is the main source of infection (39) most of which are aerobic gram positive cocci (49), including reported strains of methicillin-resistant *Staphylococcus aureus* (MRSA) (50).

When dealing with surgical antiseptics, it is necessary to talk about the type of antiseptic and the

method of application.

From a semantic point of view, it is useful to remember that disinfectant means a chemical agent with antimicrobial activity intended for the use on inanimate objects or surfaces (instrumental or environmental), whereas the antiseptic is an organic or inorganic substance used on living tissues to prevent or stop the action and growth of pathogenic microorganisms.

The ideal antiseptic should have the following properties: broad spectrum of action, rapidity of action, long duration, maintenance of effectiveness in presence of blood and organic material, good tolerability.

Chlorhexidine and povidone iodine are comparable as antimicrobial spectrum, but chlorhexidine in alcohol has a faster action, a more stable and prolonged activity and a better residual effect despite exposure to body fluids, because unlike povidone iodine is not inactivated by contact with organic substances (51). At low concentrations chlorhexidine is effective on Gram-positive bacteria, but an increase in concentration broadens the spectrum of action to include Gram-negative bacteria and fungi. Chlorhexidine is positively charged and reacts with the negative charges of the microbial cell surface, destroying the integrity of the cell membrane, penetrating inside and causing the loss of components up to cell death. Chlorhexidine has high affinity with epidermis proteins, thanks to which the molecule is adsorbed at the stratum corneum level, where it remains active for hours. The antiseptic action of iodopovidone is determined by the iodine which progressively frees itself from the complex, binds to lipids and oxidizes the components of the cytoplasm and membranes: for this reason, its speed of action is intermediate and, while using it, it is essential to guarantee an adequate contact time (in any case longer than 2'). The residual effect is scarce, and a negative aspect is that povidone iodine is rapidly neutralized by organic material. The products are on average well tolerated; iodine allergy is much more frequent than chlorhexidine allergy, but in those allergic to chlorhexidine, antiseptics with alcoholic iodopovidone is a valid second choice alternative, if applied correctly and, possibly, in 10% alcohol solution. For surgeons and surgery room staff, an added value is the presence or absence of coloring which gives security of the operating field

antiseptics (52). In general, alcohol-based antiseptic solutions are more effective than watery ones in reducing the risk of SSI, alcoholic chlorhexidine is significantly better than povidone iodine in aqueous solution in preventing superficial and deep incisional infections (53). Furthermore, iodopovidone in aqueous solution requires longer contact time, while alcoholic solutions have the advantage of drying quickly when applied to the skin, reducing the preparation time of the surgical site (22).

By comparing preparations in alcoholic solution for surgical antiseptics of patient's skin, chlorhexidine in alcohol is more effective than povidone iodine in alcohol.

A systematic review of 19 international studies showed a 30% reduction in the incidence of SSI in patients undergoing preoperative antiseptics with alcoholic chlorhexidine, compared to alcoholic iodopovidone (22), plus a meta-analysis that evaluated 13 RCT on patients undergoing clean and clean/contaminated surgery confirmed a significant superiority of the preoperative antiseptics with chlorhexidine in alcohol compared to povidone iodine in alcohol in SSI prevention (RR, 0.70, 95% CI, 0.60-0.83) (54). Chlorhexidine is more expensive than povidone iodine, but an economic analysis of studies comparing alcoholic chlorhexidine in a sterile applicator compared to alcoholic iodopovidone for surgical site antiseptics, has shown that it is up to 36% more cost-effective of alcoholic iodopovidone (55).

For mucosal antiseptics, the choice must fall on a product in an aqueous solution; in fact, alcohol and alcoholic solutions are irritating and drying for mucous membranes with the result of causing pain to the patient and paradoxically promoting the growth of microorganisms creating local damage.

Mucosal antiseptics therefore need to be evaluated not only for their antimicrobial activity, but also for any cytotoxic, irritative, sensitizing action that can be caused in different ways on different mucous membranes in different surgical areas (ophthalmology, otorino, gynecological, urological etc.).

Recent NICE guidelines state that if the surgical site is close to a mucosa, chlorhexidine in aqueous solution should be used (48).

With regard to the antiseptic product used, the regulatory aspects vary in the different countries

even if the European Chemicals Agency (ECHA) prepared a guide for the application of the European Biocides Regulation (BPR-EU Regulation 528/2012). The document specifies very clearly that products for injured skin antiseptics (e.g. surgical wound antiseptics) or intact skin antiseptics prior to invasive medical treatment (e.g. preoperative skin antiseptics before surgery or before the application of a vascular access) must always be medicinal products, and therefore fall under the regulation of Directive 2001/83/ EC (56).

Several European countries, such as Germany, the United Kingdom, Belgium and the Netherlands have already included in their national legislation the antiseptics used on skin before surgery in the field of medicinal products. According to current legislation in Italy the situation is more ambiguous: they must be registered as "medicinal products", and as such they must respond to the Legislative Decree no. 219/2006 and subsequent amendments and additions in transposition to the European Directive of antiseptics intended for use on damaged skin and mucous membranes. Instead, they can be registered at the Health Ministry as "presidi medico-chirurgici" the antiseptics used on intact skin (e.g. for staff hand washing and surgical site preparation) and disinfectants for environmental use. Finally, disinfectants for medical devices and/or equipment are registered as "medical devices".

It is not a mere classification issue, the differences and possible consequences are considerable. Drugs require clinical efficacy and safety studies for marketing and ministerial authorization (AIC), but above all, during production, the drug is subjected to Good Manufacturing Practices, there is a system production quality management, internal and external controls, a pharmacovigilance system and sterility verification. On the contrary, no specific requirements are required for biocides manufacturing process and not even sterility certificates or microbiological controls. Also in the storage and distribution chain of drugs, guarantees and checks are required from suppliers and traceability is ensured, i.e. the possibility of identifying and following a product through all stages of production, transformation and distribution up to the time of its use (and the consequent traceability, that is the possibility of following the history of

a product backwards), whereas this is not possible for biocides. The risk of bacteria or spores contamination in non-sterile antiseptic solutions, especially during the production process (intrinsic contamination), is not hypothetical, but is widely documented in literature (57,58,59) with cases of epidemics and pseudo-epidemics (60) and lots of antiseptic solutions (classified as biocides) withdrawn from the market because of contamination (61). As summarized by the Royal College of Surgeons in conjunction with the Medicines Agency, the best way to minimize damages is to use the product duly authorized for its specific intended use, in accordance with the manufacturer's instructions for use (62).

With regard to the method of application of the chosen antiseptic, the Spanish Guidelines expressly recommend a disposable applicator and a method of "back and forth" application for 30 seconds (36) which allows to reach a greater number of skin layers, going deeper into the epidermal layer and allowing a more effective reduction of bacterial load (63). It should be remembered that about 20% of bacteria live in the deepest layers of the skin, between necrotic skin cells, sweat glands and hair follicles, making adequate decontamination of the skin difficult (64). In a study comparing chlorhexidine in alcohol with applicator and iodopovidone in alcohol with gauze, with standard method, the compliance of health professionals with the protocol for the correct application of the antiseptic was significantly higher for chlorhexidine with applicator, the essential steps of the application method were completely carried out 90% of the times with chlorhexidine and 33.3% with iodopovidone ($p = 0.0001$) (65). Furthermore, since alcohol is flammable and stagnation of alcoholic solutions on patient's drapes and skin surfaces (in particular supraclavicular cavities, armpits, navel, groin...) can potentially cause a fire risk, particularly when using electrocauteries and surgical lasers (66), it is necessary to wait for the evaporation of any excess antiseptic for at least 3 minutes, or use disposable applicators to avoid shedding (41).

In summary, the disposable applicator, compared to the traditional method using gauze and multipurpose containers, improves patient's antiseptics safety, reduces risk of fire and solution contamination; in addition, standardization and practicality of the procedure is increased,

allowing to dispense the correct dose of antiseptic and reducing the risk of operator-related errors, the risk of cross-contamination and the time of application (67).

The antiseptics practice of the operating field in the obstetric-gynecological area presents particular problems related to surgery on mucous areas such as vagina or possible risks for newborn (hypothyroidism).

The frequency of cesarean sections required particular attention to the issue of preoperative skin preparation, pivotal for improving the quality of care and reducing the infection rate of the surgical site in patients undergoing cesarean section (68) (69).

In 2012 the Cochrane Library carried out a review, then updated in 2014 (70) in which 6 studies were analyzed, involving 1522 women and in which iodine, povidone iodine, alcohol, chlorhexidine and parachloromethoxylenol were used for skin preparation. As main conclusion, the authors claim that chlorhexidine gluconate shows lower bacterial growth rates after cesarean section than iodine. However, in the same conclusion the authors pointed out that in the articles there were no exhaustive data to evaluate the different antiseptics preparations in aqueous or alcoholic solution and that further studies are necessary.

In a randomized controlled trial of SSI after cesarean delivery, preoperative cutaneous antiseptics with 2% chlorhexidine and with 70% isopropyl alcohol in sterile applicator was associated with a significantly lower risk of SSI, compared to that with 8.3% povidone iodine and with 70% isopropyl alcohol (23 patients (4.0%) vs .42 (7.3%) RR 0.55, CI 95% 0.34 - 0.90). The superficial infection rate was 3.0% in the chlorhexidine - alcohol group and 4.9% in the iodine - alcohol group ($P = 0.10$); the rate of deep infection was 1.0% and 2.4% respectively ($P = 0.07$).

The reduction risk was not influenced by the fact that the caesarean section was scheduled or not, the presence or absence of obesity, the type of suture, the presence or absence of chronic diseases or the presence of diabetes. 52% of the cultures were polymicrobial and *Staphylococcus aureus* was the most commonly isolated microorganism (37%) (16). Given the higher efficacy, speed and ease of application on obstetric patients, 2% chlorhexidine in alcoholic solution in disposable applicator is of particular importance

in emergency caesarean sections where, as known, the incidence of SSI is higher and the time for fetus extraction is, in most cases, crucial.

Another extremely frequent practice in obstetrics is amniocentesis; the infection following amniocentesis is extremely rare, it is estimated 1 case per 1000 procedures for inoculation in the amniotic cavity liquid of bacterial flora present on the skin (71). For a long time, disinfection of the skin before amniocentesis was made with iodopovidone-based solutions (72), where chlorhexidine was superior as skin antiseptic in the positioning of venous catheters and before blood culture samples (73). In a study of 50 pregnant women, the skin was cleaned with chlorhexidine in alcoholic solution on one side and povidone iodine on the other. The post-cleaning colony average count was 17.3 for povidone iodine and 0.12 for chlorhexidine, the median for povidone iodine was 2 (range from 0 to 142), whereas for chlorhexidine in alcoholic solution it was 0 (range from 0 to 3) with a statistically significant results in favor of chlorhexidine. In the chlorhexidine group colonies developed only in 3 out of 50 plates, whereas in the iodopovidone group colonies developed in 30 plates out of 50 (72).

In a retrospective study, two homogeneous groups of patients undergoing elective laparoscopic gynecological surgery were compared in which the skin antiseptics was made with iodopovidone or with chlorhexidine in alcoholic solution. The infection rate was 14.6% in the iodopovidone vs. group. 4.5% in the chlorhexidine in alcoholic solution group ($p = 0.011$) with a risk of developing SSI 3.2 times higher (95% CI 1.13-9.30) in the iodopovidone group compared to chlorhexidine in alcoholic solution. Obviously, hospitalization in patients with SSI was significantly longer (11.8 ± 7.7 vs. 6.3 ± 3.2 , days, $p = 0.002$) (74).

Conventional skin antiseptics usually leaves more product than necessary on the skin, which accumulates in the sloping areas, and can wet the sheets which will then remain in contact with the patient's hips or back with the risk of causing chemical burns. The application of the exact dose obtained with 2% chlorhexidine in 70% isopropyl alcohol in sterile applicator will avoid this risk, particularly important in oncologic surgery, which have rather long surgery time such

as in cervical, endometrial and ovarian cancer surgery (36).

Pelvic floor surgery treats an area where skin and mucous surfaces coexist.

Vaginal delivery is physiological and the birth canal is not currently cleaned; in the past the risk of inducing neonatal hypothyroidism was demonstrated with the use of iodine-based preparations (75), and therefore aqueous chlorhexidine was preferred.

In a randomized comparison study between 4% chlorhexidine gluconate and 10% iodopovidone in patients undergoing vaginal hysterectomy, the cultures taken at 30 minutes in the iodopovidone group were 6 times more likely to be contaminated than those taken in the chlorhexidine group, a trend similar is confirmed for crops obtained at 90 minutes even if the difference does not reach statistical significance (76). Chlorhexidine as a vaginal preparation has shown to be safe in 3 large randomized studies (77,78,79), but a case of desquamatory reaction after vaginal scrub with chlorhexidine has also been reported (80).

Iodopovidone (10% iodine) in aqueous solution is currently used whenever vagina asepsis is needed, such as before the manipulator insertion in laparoscopic surgery.

INTRAOPERATIVE MANAGEMENT

Tissue Oxygenation

Patients undergoing general anesthesia with endotracheal intubation and mechanical ventilation must receive oxygen supplementation during surgery and, if possible, in the immediate postoperative time (for 2-6 hours) in order to reduce the risk of SSI.

(WHO 2016, strong/moderate; CDC 2017, IA). According to WHO Guidelines, the value of the inspired oxygen fraction (FiO_2) should be 80% (WHO 2016, strong / moderate).

A meta-analysis conducted by the WHO Guidelines expert group has shown that the perioperative oxygen inspired fraction (FiO_2) 80% is superior to the standard (30-35%) in reducing the risk of SSI in patients undergoing surgical procedures under general anesthesia with endotracheal intubation and through a high flow mask in the immediate postoperative time.

Oxygen supplementation beyond the standard

of 30% is intended to improve tissue oxygenation in the surgical area and to enhance the patient's defense by increasing the oxidative capacity of the neutrophils.

The effect of hyperoxygenation is maximized by the maintenance of perioperative normothermia and normovolemia.

Normothermia

It is recommended to keep patient's normothermia throughout the perioperative time. (WHO 2016, conditioned/moderate; CDC 2017, IA):

- the use of heating devices in the surgery room and during the surgical procedure to avoid patient's hypothermia is suggested in order to reduce SSI risk and other important complications (myocardial events, blood loss requiring transfusions) (WHO 2016, conditioned/moderate).

No recommendation can be made on what is the best system to obtain and maintain normothermia, on minimum temperature level to be reached, on best time to start heating and optimal duration (CDC 2017, no unresolved recommendation/topic).

Maintenance of normothermia has a significant benefit on reducing the risk of SSI; a decrease in body temperature can occur commonly during surgery due to a reduced function of neutrophilic granulocytes and secondary hypoxia to vasoconstriction, both associated with an increased risk of SSI (41).

From a practical point of view, the patient at high risk of hypothermia should be identified (ASA from II to IV, temperature <36 ° C before urgent surgery, combined general and loco-regional anesthesia, major or intermediate surgery, risk of cardiovascular complications) (41).

Particular attention must be paid in long-term surgeries.

Normoglycemia

Adequate perioperative glycemetic control is recommended in all surgical patients, diabetic and

non-diabetic, in order to reduce the risk of SSI.

(WHO 2016, conditioned / low; CDC 2017, IA):

- the benefit of a glycemetic target < 200 mg/dl is certain (CDC 2017, IA).
- Low quality evidence supports additional benefits associated with intensive glycemetic control (<110-150 mg/dl) (WHO 2016, conditioned/low).
- In case of glycemetic target <110 mg/dl, pay attention to the risk of hypoglycaemia (WHO 2016).

Studies towards an intensive glycemetic control both in intra- and in postoperative phases showed advantages in SSI onset.

Surgical stress causes an increase in blood glucose levels during and after surgery and several observational studies have shown that such hyperglycaemia is associated with an increased risk of SSI in both diabetic and non-diabetic patients, in different types of surgery (81).

Normovolemia

Monitor patient's blood volume throughout the intraoperative time and ensure adequate restoration of body fluids (WHO 2016, conditioned/low; CDC 2017, IA):

- the intraoperative use of Goal Direct Fluid Therapy (GDFT) protocol is recommended in order to reduce the SSI risk (WHO 2016, conditioned/low).

The intraoperative GoalDirectFluidTherapy protocol has a significant benefit in reducing the rate of SSI compared to standard fluid management and the effect is confirmed in the postoperative time. The GDFT protocol allows to standardize patient's hemodynamic monitoring by evaluating the volume based on volumetric parameters such as the Stroke Volume (SV) or the Stroke Volume Variation (SVV), rather than pressometric, in order to restore an adequate state of hydration and maximize the release of oxygen to the tissues (82).

The control of blood losses must be particularly careful in obstetrics, if there is fluid loss more than 500 ml, fibrinogen is administered.

OBSTETRIC-GYNECOLOGICAL APPLICATIONS

150 years after Ignazio Semmelweis's fundamental observations on puerperal sepsis and its reduction due to the antiseptic of the surgeons' hands, SSI is now at the center of a renewed interest because surgical procedures are increasingly complex, and candidate patients have a much higher risk of contracting infections than in the past (more advanced average age, polymorbidity, even debilitated subjects, polypharmacies).

In the obstetric-gynecological field, surgeries of greatest interest for SSI are those in obstetrics, in oncological surgery and urogynecology; fortunately, minimally invasive techniques have reduced, but not eliminated, the infectious risk. The problem is however of great importance because in Italy 45% of germs are multi resistant.

The proposed actions are recommended with the aim of reducing the impact of surgical site infections in terms of morbidity and mortality. To implement them, plans and investment in human capital are required by the health organization because the action effectiveness does not depend on their formal correctness, but on the ability to make them concrete in the daily context.

The implementation of evidence-based bundles (definable as the implementation of three or more processes capable of preventing SSI) for the prevention of infections in cesarized patients has shown, in a broad meta-analysis, to be able to lower the infection rate from 6.2% to 2.0% (pooled RR 0.33, 95% CI 0.25-0.43) (83).

The change in previously assumed, often consolidated, behaviors requires determination and a clear responsibility of each health professional. Conscious adherence comes from a correct information and training (to explain the reasons of some choices, the assess of surgery feasibility and how to memorize them) and it is followed by a process of validation test of the correct execution and surveillance activity results in order to increase awareness among health professionals about the importance of preventing SSI and their role in ensuring patient safety. The training program must also include periodic educational reinforcement updates and audit programs (84).

In the training process, checklists must be introduced that, summarizing the most important evidence and translating it into concrete behaviors, become an important tool for disseminating updated knowledges in clinical practice, for standardizing work processes and systematic controls related to key processes (85) as well as having important and useful legal value.

In addition to training and people motivation, maximum connection and structured communication among healthcare team components taking care of patients in the pre-, intra- and post-operative phase is mandatory. Implementation of actions to fight SSI in the obstetric-gynecological field not only affects surgical acts, but the system as a whole and also involves healthcare professionals external to the surgical room: for example, the microbiological report is part of a treatment path in case of SSI and represents the opportunity for a professional relationship between the microbiologist and the other healthcare professionals, the presence of a clinical pharmacologist allows an in-depth knowledge of pharmacokinetics and pharmacodynamics molecules interacting with surgical patients. Furthermore, the active involvement of managers whose conviction and endorsement are used to motivate staff and redefine processes by ensuring appropriate infrastructures and technological equipment is also essential, with the awareness that the additional cost of prevention can in many cases be more than compensated by savings deriving from the SSI reduction and its costs associated. An example is given by the widespread use of disposable sterile supplies, such as drapes, gloves and staff clothing, but also trichotome heads and disposable applicators for skin antiseptic (67). Technological innovation always offers new contributions in reducing SSI risks, such as suture threads with antibacterial agents (triclosan) (48).

A fundamental aspect in prevention consists in guaranteeing specific communication strategies with patients involved in the preparatory phase, remembering how high the motivation of obstetric patients is, while in gynecology is on average lower. SSI studies show the importance of post-discharge surveillance in assessing the size of the problem, which can be implemented by introducing the practice of calling patients at home

to check their progress. Particular attention requires communication to women belonging to the so-called "Hard to reach populations" who will be able to use cultural mediators, multilingual supplies, etc.

With the opportunity of SSI prevention, SIGO draws the attention to the surgery room environment, not only regarding the patient temperature, but also to the surgeon's comfort.

It would be necessary to request the attention of all the surgical room staff in keeping the doors closed, avoiding crowding and noise to reduce the possibility of surgeons' error. In this regard, the robotic room is particular because often the room staff tends to be distracted.

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Polycystic Ovary Syndrome in adolescents: an update

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ABSTRACT

Menstrual disorders and evidence of hyperandrogenism are characteristic signs of polycystic ovary syndrome (PCOS) in Teens. Diagnosis of PCOS is difficult as clinical signs that distinguish it can be also found in the general population. Specific etiology of PCOS is unknown but it is a complex disease that results in genetic, intrauterine, ectopic and environmental factors. Obesity and insulin resistance are factors commonly associated with PCOS and, as a result, patients are at risk of metabolic and cardiovascular diseases.

Changes lifestyle and drug therapy are recommended in all patients in order to control and counteract development of hyperandrogenism and menstrual disorders.

SOMMARIO

I disordini mestruali e il riscontro di iperandrogenismo sono segni caratteristici della sindrome da ovaio policistico (PCOS) negli adolescenti. La diagnosi di PCOS è difficile in quanto i segni clinici possono essere riscontrati anche nella popolazione generale. L'etiologia specifica della PCOS è sconosciuta ma si tratta comunque di una malattia complessa in cui sono coinvolti fattori genetici, intrauterini, ectopici ed ambientali. Obesità ed insulinoresistenza sono fattori comunemente associati alla PCOS e, di conseguenza, i pazienti sono ad alto rischio di malattie metaboliche e cardiovascolari. Modifiche allo stile di vita e terapia farmacologica sono raccomandate in tutte le pazienti al fine di controllare e contrastare lo sviluppo di iperandrogenismo e disordini mestruali.

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Key words: PCOS, adolescents, hyperandrogenism

INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 3.5-15% women in reproductive age (1,2). It is the most common cause of hyperandrogenism and anovulatory infertility in this age group (3,4). Characteristic clinical signs in women are:

- menstrual irregularities (oligomenorrea or amenorrea),
- clinical hyperadrogenism (hirsutism, acne, androgenic alopecia and anovulatory infertility) (5).
- Rarely, women can present virilisation(6).

Diagnostic criteria used for PCOS have been changes over time as shown in **Table I**.

Table I. Diagnostic criteria used for PCOS over time

1990	- National Institute of Health: PCOS defined as clinical or biochemical hyperandrogenism and oligo-anovulation in the absence of other endocrinopathies (7).
2003	- Rotterdam consensus: broadening the criteria to include the presence of at least two of the following as diagnostics for PCOS: 1. Biochemical or clinical Hyperandrogenism, 2. Oligo-anovulation, 3. Polycystic ovarian morphology to ultrasound examination (PCOM)(8).
2012	- NIH: confirmed Rotterdam 2003 criteria for PCOS diagnosis but are to be specified different phenotypes (9).

PCOS is an heterogeneous disease in terms of both pathophysiology and severity of clinical consequences. Not all patients show all clinical signs or are exposed to the same degree of long-term health risk.

The adoption of the Rotterdam criteria, and in particular the decision to consider two of the three typical elements of the syndrome sufficient for diagnosis, has resulted in different clinical presentations within the same PCOS name, the so-called PCOS phenotypes:

- "classic": when oligoanovulation and hyperandrogenism are present, with or

without the micropolycystic aspect of the ovary,

- "ovulatory": when hyperandrogenism and micropolychistic aspect are present,
- "normoandrogenic": in presence of oligoanovulation and micropolycystic aspect.

In this subdivision, the NIH also recommends distinguishing within the classical phenotype subjects with or without ovarian micropolycystic appearance. The former, therefore, have all the manifestations of the syndrome, but it is not clear whether a difference limited to ovarian morphology alone implies real physiopathological and clinical differences.

Women with phenotype A (hyperandrogenism and oligomenorrhoea) present a higher risk than non-hyperandrogenic PCOS.

PATHOGENESIS

The exact aetiology of this disorder is yet to be clarified but insulin resistance and compensatory hyperinsulinism have been seen, however, to play a central role in the pathogenesis (10). As already mentioned, it is a complex multifactorial disease with an interaction of several factors: genetic predisposition, insulin resistance, dysfunction of beta pancreatic cells, abnormal ovarian and adrenal steroidogenesis and alterations of steroid metabolism, neuroendocrine influences, environmental factors, epigenetic mechanisms and abnormal adaptation to reduction or excess of energy.

Hyperandrogenism

PCOS is primarily an ovarian disease with increased androgen production. The cells of the theca produce androgens under control of LH and various intracrine factors. P450c17 is an enzyme that plays a central role in the synthesis of androgens. Its increased expression, as well as the enzyme CYP17A1, is evaluable in the cells of the theca of women with PCOS (11). Hyperinsulinemia, associated with PCOS, exaggerates the response of the cells of the theca to circulating LH. Ovaries with PCOS also have an increased expression of enzymes involved in the

production of dihydrotestosterone (12).

In patients with PCOS there are also increases in androgens 11-oxygenates, 11 beta hydroxyandrostenedione, 11 ketoandrostenedione, 11 beta hydroxytestosterone, 11 ketotestosterone. In 20-30% of women, DHEA and DHEA sulphate are increased.

Insulin-Resistance

Insulin resistance and hyperinsulinemia play a central role in the pathogenesis of PCOS with a high prevalence found in women and adolescents with PCOS (13).

Regardless of the BMI, this disorder affects thin and obese women.

Insulin resistance in patients with PCOS is determined due to complex interactions involving genetic predisposition, intrauterine factors, early puberty and adiposity.

Insulin resistance is selective tissue and is present in muscle, liver and adipose tissue, mainly due to the metabolic effects of insulin.

Obesity is the most common sign of PCOS. Obesity, especially abdominal obesity, worsens the clinical and hormonal picture of PCOS. It is reported that about 30-40% of adolescents with PCOS are overweight or obese (14,15). Despite the high prevalence, however, obesity or insulin resistance are not included in the diagnostic criteria of PCOS. However, these confer a high risk of various metabolic abnormalities such as type 2 diabetes, hypertension, dyslipidaemia or metabolic syndrome in these patients.

Genetics

Familial clustering of hyperandrogenism, anovulation and PCO suggest an underlying genetic basis or cause. Studies in large families have suggested autosomal dominant inheritance, with premature balding as the male phenotype. Other studies of siblings and parents of women with PCOS have observed high prevalence of hyperinsulinemia and hypertriglyceridemia, PCOS in females and premature balding in males.

Nearly 50% of sisters of women with PCOS have elevated total or bioavailable testosterone concentrations and approximately 35% of mothers also are affected. The first degree relatives of women with PCOS also exhibit other metabolic abnormalities such as dyslipidemia, which may predispose to an increased risk for cardiovascular disease. These observations further suggest a genetic predisposition or susceptibility.

The genetic component contributes to 70% of the pathogenesis of PCOS.

Genome wide association studies (GWAS) (16,17) have begun to identify several possible candidate genes, including the DENND1A (differentially expressed in normal and neoplastic cell domain-containing protein 1A) and THADA (thyroid adenoma-associated protein) genes. Further support for DENND1A comes from studies demonstrating increased levels of the V2 isoform of DENND1A in theca cells obtained from women with PCOS (18). Currently, PCOS is best seen as a polygenic disorder involving the interaction of numerous genomic variants and the influence of environmental factors. Candidate genes include the long list of molecules (Table II) that participate in any of the metabolic and reproductive pathways affected in the syndrome, emphasizing yet again that PCOS is not a specific endocrine disorder, but a result of chronic anovulation due to a wide variety of causes.

DIAGNOSIS OF PCOS

Hyperandrogenism

Hyperandrogenism in teens is related to the development of hirsutism, acne, menstrual irregularities and alopecia. Hirsutism is characterized by the growth of terminal hairs in androgen-dependent areas with patterns similar to those of men. The prevalence varies with ethnicity, being greater in Mediterranean, Caucasian white, Hispanic and Middle Eastern women (19), lesser in Chinese women (20). Alopecia is less common in teenagers.

Acne comedonale is, instead, common in adolescents (21). Where this symptom is resistant to

Table II. Genetics of PCOS

Genes	Chromosome	Function
LHCGR	Chromosome 9	Gonadotropin action
FSHR	Chromosome 2	Gonadotropin action
THADA	Chromosome 2	Apoptosis
DENND1A	Chromosome 2	Endocytosis, receptor-mediated turnover
YAP1	Chromosome 11	Cell proliferation
GATA4	Chromosome 8	Transcription factor
C9orf3	Chromosome 9	Aminopeptidase
HMGA2	Chromosome 12	Transcriptional regulated factor
TOX3	Chromosome 16	Chromatin remodeling
INSR	Chromosome 19	Insulin signaling
SUMO1P1	Chromosome 20	Cell proliferaton
KCNA4/FSHB	Chromosome 11	Gonadotropin action
ERBB4	Chromosome 2	Mitogenesis and differentiation
RAD50	Chromosome 5	DNA repair
KRR1	Chromosome 12	Ribosome biogenesis
RAB5B, SUOX	Chromosome 12	Protein transport, breakdown

therapy, the suspicion of hyperandrogenism must be placed.

Biochemical hyperandrogenism is documented by free or total testosterone. In addition, about 10-15% of patients, may have an increase in adrenal androgens, DHEA-S or Androstenedione. The detection of menstrual irregularities in adolescence, in the early years of the menarche, is very common. The average age of the menarche in the population is 12-13 years. In the first year after the menarche, 60% of girls have 10 cycles per year and this can last up to 5 years. The finding of amenorrhoea or menstrual cycles > 90 days after one year from the menarche should suggest hyperandrogenism.

In adolescence, chronic anovulation may also be common in girls with dysfunctional uterine bleeding, although this may also occur in regular cycles.

The finding of a low to medium serum luteal progesterone level confirms anovulation. According to international guidelines, oligo-anovulation must be considered if menstrual intervals shorter than 20 or longer than 45 days persist 2 years after the menarche.

PCOS diagnosis should be considered in girls with final height obtained and puberty

development but who have primary amenorrhea.

Polycystic ovarian morphology (PCOM)

The Rotterdam criteria in 2003 included PCOM as one of the criteria for the diagnosis of PCOS. On TV ultrasound examination, the ovaries of women with PCOS, in addition to having many small follicles, have an increase in size and stromal volume as well as a classic topographic relationship between the follicles and the stroma. The many small follicles are arranged peripherally and surround a dense central stroma of a polycystic ovary.

According to The Rotterdam consensus criterion, PCOM is characterized by the presence of ovaries with a volume > 10 mL³ and the presence of a total number of small follicles ranging in size from 2 to 9 mm for a number equal to or greater than 1222 (**Figures 1-2**); the presence of at least one of these two morphological ultrasound signs in an ovary serves as a meeting the Rotterdam Criteria for polycystic ovary. The prevalence of PCOM in adolescents is 35-40%²³. The prevalence of PCOM is quite high in women with hyperandrogenemia (> 80%). Despite this, however, both in one on three cases of women

with regular menstrual cycles without PCOS, and in 14% of women who use oral contraceptives you can find PCOM.

In addition, PCOM is commonly observed during normal adolescence and in women with hypothalamic amenorrhea and hyperprolactinemia. PCOM should not be confused with a multi-follicular pattern defined by the presence of more than 6 follicles of 4-10 mm distributed in the ovary.

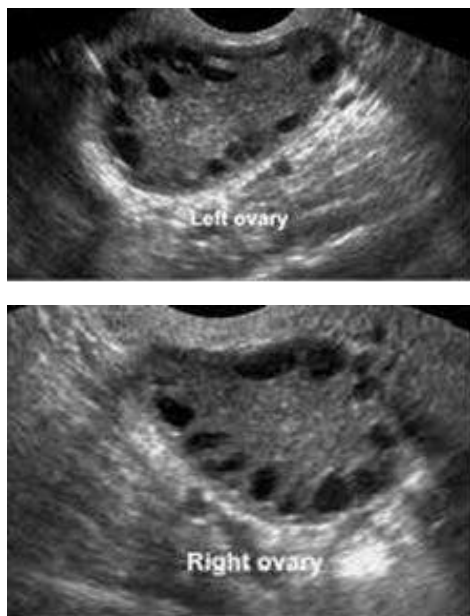


Figure 1. Ultrasound features of the PCOS.

Other features of the PCOS

PCOS has other common features besides hyperandrogenism and ovulatory dysfunction that are not included in any diagnostic criteria, including:

- abnormal of gonadotropin secretion,
- insulin resistance and related metabolic abnormalities (p.e. dyslipidemia)

EXCLUSION OF OTHER ANDROGEN EXCESS DISORDERS

The diagnosis of PCOS is secondary to the exclusion of other pathological patterns responsible

for oligo-anovulation and hyperandrogenemia (Table III).

These conditions are:

Thyroid disorders

- Hyperprolactinemia
- Cushing's syndrome
- Non classical congenital adrenal hyperplasia
- Ovarian or adrenal secrete androgenic tumors.

Table III. Differential Diagnosis with other androgen excess disorders.

Diagnosis	Method of exclusion
Thyroid disorders	serum thyroid-stimulating hormone, TSH
Hyperprolactinemia	Serum PRL
Nonclassical Congenital Adrenal Hyperplasia	a follicular phase morning serum 17-OHP concentration less than 200 ng/dL
Androgen Secreting Ovarian and Adrenal Tumors	serum total testosterone concentration greater than 150 ng/dL
Cushing Syndrome	overnight dexamethasone suppression test

1. Thyroid disorders

They are associated in teens with menstrual dysfunction. The overall high prevalence of thyroid dysfunction in teens warrants specific testing to exclude the diagnosis (serum thyroid-stimulating hormone, TSH) in all anovulatory girls, including those with hyperandrogenism, but not for diagnosis of PCOS.

2. Hyperprolactinemia

The high prevalence of hyperprolactinemia among girls with menstrual dysfunction justifies specific testing to exclude the diagnosis in all anovulatory girls, but not for diagnosis of PCOS.

3. *Nonclassical Congenital Adrenal Hyperplasia*

Congenital adrenal hyperplasia (CAH) is caused by adrenal steroidogenic enzyme defects that result in excessive adrenal androgen production. The most common cause is 21-hydroxylase deficiency. Female with nonclassical or "late-onset" form of CAH present during childhood or early adolescence with precocious puberty, or as young adults with signs of hyperandrogenism, very much like those with PCOS. Whereas it is logical to recommend that nonclassical CAH be excluded in all women with hyperandrogenism, specific testing can be also reserved in case of early onset of hirsutism (pre- or peri-menarchal, including girls with premature adrenarche) girls with a family history of the disorder, and those in high risk ethnic groups (Hispanic, Mediterranean, Slavic, Ashkenzi Jewish). Estrogen-progestin contraceptives and/or antiandrogens are the best choice for the treatment of chronic anovulation and hirsutism in women with nonclassical CAH.

A follicular phase morning serum 17-OHP concentration less than 200 ng/dL excludes, and a level greater than 800 ng/dL establishes the diagnosis. Concentrations between the two threshold values suggest the possibility, which can be confirmed by performing an ACTH stimulation test, obtaining blood samples before and 60 minutes after administering synthetic ACTH im or iv.

4. *Androgen Secreting Ovarian and Adrenal Tumors*

These are rare events. The possibility of a tumor is excluded by the clinical history and physical examination. A serum total testosterone concentration greater than 150 ng/dL, identifies almost all women with a potential androgen-producing tumor. However, a tumor should be suspected also in girls with rapidly progressive hirsutism, even with a low level of serum testosterone. Transvaginal ultrasonography will identify almost all solid ovarian mass lesions. Adrenal computed tomography (CT) is extremely

sensitive for detecting rare androgen-secreting adrenal tumors.

5. *Cushing Syndrome*

It results from excess adrenal cortisol secretion and can be ACTH dependent (pituitary and ectopic ACTH-secreting tumors) or ACTH-independent (adrenal adenomas, exogenous glucocorticoid treatment). The disorder has features commonly observed in girls with PCOS (menstrual dysfunction, hyperandrogenism, central obesity). The overnight dexamethasone suppression test is the best single screening test to discriminate. The test is performed by administering 1.0 mg of dexamethasone between 11.00 pm and midnight and measuring the serum cortisol at 8 am the next morning; values less than 1.8 µg/dL are normal.

ASSOCIATED COMORBIDITIES

The health consequences of PCOS are linked to obesity, insulin resistance and hyperandrogenemia. There is an increased prevalence of glucose intolerance and diabetes (24), metabolic syndrome (25) and cardiovascular disease (26). All adolescents should undergo blood pressure measurement, glucose load tests and lipid profile (27). The follow up is to be repeated annually in overweight/obese adolescents, with familiarity with diabetes and cardiovascular disorders.

THERAPY

Lifestyle intervention

The strong association between obesity, hyperandrogenism and menstrual abnormalities emphasizes the importance of addressing lifestyle issues in women with PCOS, focusing on nutrition and exercise. At least 50% of women with PCOS are obese. It is important to stress that even a small reduction in weight (2-5%) can result in significant improvements in metabolic and reproduction function. The loss of abdominal fat may be the best predictor of the effects of weight loss.

Weight reduction is the first-line management strategy for overweight and obese teens. Weight loss increases SHBG, thereby reducing free androgen levels and decreasing androgen stimulation of the hair and skin. Weight loss also improve ovulatory function.

The benefits of exercise for improving cardiovascular health and decrease risk of diabetes have been demonstrated in the general population. Incorporation of moderate activity into daily activities appears as effective for reducing the risk of developing diabetes and cardiovascular disease as that achieved with vigorous physical activity is more likely to be sustained and is essential for maintaining weight loss over time²⁸⁻³⁰.

Combined oral contraceptive therapy

Combined oral contraceptives (COCs) should be considered a first-line drug in adolescents with a certain diagnosis of PCOS for the menstrual abnormalities associated with chronic anovulation²⁷. COCs decrease adrenal and ovarian androgen production and reduce hair growth in nearly two-thirds of hirsute patients. Treatment with OCs offers the following benefits:

1. The progestin component suppress LH, resulting in diminished ovarian androgen production.
2. The estrogen component increases hepatic production of SHBG, resulting in decreased free testosterone concentration
3. Circulating androgen levels are reduced, including these of DHEAS.
4. Estrogens decrease conversion of testosterone to DHT in the skin by inhibition of 5 alpha reductase.

Low-dose estrogen or natural estrogen are preferred to reduce the negative action on the metabolic profile, the increase in triglycerides and insulin resistance and increased VTE risk.

Medroxyprogesterone Acetate (MPA)

Oral or intramuscular administration of MPA successfully treats hirsutism. It directly affects

the hypothalamic-pituitary axis by decreasing GnRH production and the release of gonadotropins, thereby reducing testosterone and estrogen production by the ovary. Despite a decrease in SHBG, total and free androgen levels are decreased significantly.

The recommended oral dose for GnRH suppression is 20 to 40 mg daily in divided dosage or 150 mg given intramuscularly every 6 weeks to 3 months in the depot form. hair growth is reduced in up to 95% of patients. Side effects of the treatment include: amenorrhea, depression, bone mineral density loss, headaches, fluid retention, hepatic dysfunction and weight gain. MPA is not commonly used for hirsutism

Antiandrogens

Antiandrogens are effective for the treatment of hirsutism but must be used in combination with highly reliable contraceptive method (e.g. ad intrauterine device) because of their potential to adversely affect sexual development in a male fetus if the patient were to conceive unexpectedly.

Spironolactone is the most commonly used as antiandrogenic drug. The most common dose is 50 to 100 mg twice daily. Women treated with 200 mg for day show a greater reduction in hair shaft diameter than women receiving 100 mg per day. Maximal inhibition of hirsutism is noted between 3 and 6 months but continues for 12 months. The most common side effect of spironolactone is menstrual irregularity (usually metrorrhagia), which may occur in over 50% of patients with a dosage of 200 mg per day. Infrequently other side effects such as mastodynia, urticaria, scalp hair loss may occur.

Other option commonly utilized in clinical practice include *finasteride*. Most of the improvement in hirsutism with finasteride occurs after 6 months of therapy with 7,5 mg per day. OCs in combination with finasteride are more effective in reducing hirsutism than finasteride alone. It is well tolerated, with minimal hepatorenal toxicity.

Flutamide, a pure nonsteroidal antiandrogen, in

hyperinsulinemic, hyperandrogenemic, non-obese PCOS adolescents on a combination of metformin (850 mg per day) and flutamide (62,5 mg per day), and the low-dose OC containing

The combination of echini-drospirenone, metformin and flutamide is effective in reducing excess total fat, abdominal fat, and attenuating dysadipocytokinemia in young women with hyperinsulinemic PCOS. Flutamide is poorly used because it is hepatotoxic even if the dose of 1 mg/kg/day is not hepatotoxic (31).

Eflornithine is a reversible inhibitor of ornithine decarboxylase, an enzyme that is needed for cell division and eflornithine hydrochloride is available as a topical treatment that, if applied regularly, is effective in slowing the growth of unwanted facial hair.

Metformine

It is an insulin sensitizer that has shown beneficial effect on weight, metabolic parameters especially glucose tolerance and menstrual irregularity in adolescents (29,32).

Most of the studies are limited by small sample size and short study duration (usually 16-24 weeks).

No serious adverse events have been reported.

drospirenone, resulted in a more effective and more efficient reduction in total and abdominal fat excess than was demonstrated by those utilizing an OC with gestodene as the progestin.

The recent guidelines state that the metformin, in addition to lifestyle interventions, can be used in adolescents diagnosed with PCOS or PCOS symptoms before reaching a certain diagnosis.

SUMMARY

PCOS is a heterogeneous condition with hyperandrogenism and menstrual irregularity as its key signs. The exact aetiology of this condition remains unknown. Diagnosis of PCOS in adolescence is difficult because clinical signs of PCOS cannot be differentiated from physiological conditions of puberty. Obesity, insulin resistance, hyperinsulinemia are common associated and play a role in the pathogenesis. Because these patients have a high risk of developing diabetes, dyslipidemia, hypertension and cardiovascular disease, lifestyle changes are recommended in all patients with PCOS.

Oral contraceptives, antiandrogenic drug and metformin alone or in combination, are used to treat clinical signs of hyperandrogenemia.

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The adherence adequacy to antenatal care in alleviating the adverse maternal and neonatal outcomes of Iranian pregnant women: a retrospective-prospective study

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ABSTRACT

Background. Low adherence of pregnant women to the content of prenatal care guidelines (PCGs) is one of the most significant issues in obstetrics.

Objective. This study aimed to improve the well-being of the mother and fetus by preventing maternal and neonatal complications using the adherence to antenatal care (ANC).

Patients and methods. An integrated retrospective-prospective longitudinal study with participating 604 pregnant women was carried out in the Iranian community. A valid researcher-made tool was used to implement this three-stage research approach. The results of demographic and obstetric characteristics, the adequacy of adherence to PCGS, and pregnancy outcomes were analyzed using multivariate logistic regression to assess the compliance of women with pregnancy outcomes.

Results. 71.36% of the total population had a complete adherence ($\geq 80\%$) to the PCGs. The women with higher education levels substantially showed more adherence to this healthcare program guideline ($p = 0.0001$). In relation to pregnancy outcomes, there was a significant relationship between women's adherence to PCGs and neonatal complications ($p < 0.05$).

Conclusion. The assessment of PCGs content was successful in evaluating ANC quality. The complete adherence to first ANC guidelines could efficiently promote neonatal outcomes, whereas it showed no effect on maternal outcomes.

SOMMARIO

Background. La scarsa aderenza delle donne incinta al contenuto delle linee guida per l'assistenza prenatale (PCG) è una delle questioni più significative dell'ostetricia.

Obiettivo. Questo studio mira a migliorare il benessere della madre e del feto, prevenendo le complicazioni materne e neonatali con l'aderenza alla cura prenatale (ANC).

Pazienti e metodi. Nella comunità iraniana è stato condotto uno studio longitudinale retrospettivo/prospettivo integrato con 604 donne incinta partecipanti. Per implementare in tre fasi questo approccio di ricerca è stato utilizzato un valido strumento per ricercatori.

I risultati delle caratteristiche demografiche e ostetriche, l'adeguatezza dell'aderenza al PCGS e gli esiti della gravidanza sono stati analizzati utilizzando la regressione logistica multivariata, al fine di valutare la conformità delle donne agli esiti della gravidanza.

Risultati. Il 71,36% della popolazione totale ha avuto una completa aderenza (80%) ai PCG. Le donne con livelli di istruzione superiore hanno mostrato sostanzialmente una maggiore aderenza a questa linea guida del programma sanitario ($p = 0,0001$). Per quanto riguarda gli esiti della gravidanza, c'è stata una relazione significativa tra l'aderenza delle donne ai PCG e le complicazioni neonatic ($p < 0,05$).

Conclusione. La valutazione dei contenuti dei PCG è risultata efficace per la valutazione della qualità dell'ANC. La completa adesione dell'ANC alle prime linee guida potrebbe promuovere efficientemente i risultati neonatali, mentre non ha mostrato alcun effetto sui risultati materni.

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Key words: maternal adherence; prenatal care; maternal and neonatal mortality; pregnant women; pregnancy outcome.

INTRODUCTION

Antenatal care (ANC) is one of the most recommended measures to prevent pregnancy complications and one of the primary interventions in improving care and neonatal and maternal conditions. ANC services establish proper communication between service providers (physicians and obstetrics) and pregnant women through enabling early diagnosis of risks and complications associated with pregnancy and ensuring access to health services such as health education, vaccination, and diagnostic and therapeutic tests (1,2). A leading and long-standing issue in prenatal care is the timing, content, and type of care during ANC visits. Therefore, purposes of ANC are to deliver healthy newborn babies with a minimum level of maternity risks through the determination of gestational age, recognition of maternal risks, persistent assessment of the health status of the mother and fetus, prognoses and essential interventions, establishing the proper doctor-patient communication, and offering the necessary education (3-7).

Despite improvements in prenatal care, about 800 women and 7700 newborns die every day due to complications in pregnancy, childbirth, and postpartum in the world (8,9). Based on the statistics released by Ministry of Health of Iran, the maternal mortality ratio (MMR) in Iran has been reported to be 23 cases per 100,000 live births in 2013. Although the MMR in this country has further reduced to 20.9% in 100,000 live births in 2017, this ratio still seems to be high (10). In any cultural and socio-demographic context, women need to have a positive pregnancy experience with high quality of antenatal and postnatal cares. Utilizing the prenatal care is important to prevent undesirable pregnancy outcomes. Studies have shown that there is an association between later initiation of prenatal care or having less than four prenatal visits and adverse fetal outcomes such as birth weight or preterm birth (6,9).

There are some differences in the national ANC recommendations among different countries concerning the number, timing, and quality of the visits. However, the WHO recommended a minimum of four visits for the pregnant women with uncomplicated pregnancies, so that the first visit should be before the 14th week of gestation. Moreover, it was mentioned that four ANC

visits on the 4th, 6th or 7th, 8th, and 9th pregnancy months are necessary for women with normal pregnancies in low- and middle-income countries (9-11). Women with high-risk pregnancies experience negative psychiatric feelings like fear, fury, loneliness, disappointment, and hope (16). Accordingly, the ANC guideline of WHO for women with high-risk pregnancies includes three specific policies: (i) routine care (offered to all women and babies), (ii) additional care (for women and babies with moderately-severe diseases and complications), and (iii) specialized obstetrical and neonatal care (for women and babies with intense diseases and complications) (11-14).

One of the major problems in many specialties, particularly obstetrics is low women's adherence to the related health protocols. Lower adherence to prenatal care has been found to cause lower 5-minute Apgar scores, higher rates of perinatal death, intrauterine infection, and placental abruption, and increased admissions of infants in the neonatal intensive care unit (NICU) (15). Although ANC, as the significant healthcare intermediary has widely been accepted, its efficiency and efficacy in improving the maternal and neonatal outcomes have less been remained unproven. This study aimed to evaluate the adequacy of ANC and its association with pregnancy outcomes, using an approach based on the assessment of the content adequacy and Iranian women's adherence to prenatal care guidelines (PCGs).

METHODS

Study design and participants

A retrospective/prospective longitudinal study was conducted in Tehran's 3rd level hospitals between February and July 2018. These hospitals had surgical, internal, gynecological, pediatric, anesthetic, neurological, infectious, radiological, pathological, and laboratory specialties and were equipped with intensive (ICU), coronary (CCU), and medical intensive (MICU) care units, and NICU. All high-risk pregnant women in hospitals were referred to these care units in the private and public sectors. A convenient sampling method was used to recruit 604 pregnant women between 24 to 34 weeks of gestation,

who participated in this research plan. All the pregnant women were categorized into two groups of low-risk and high-risk based on the interview and case history information. The sample size using a 95% confidence interval, and power of 80% was estimated by an Open-Epi calculator based on the prevalence of pregnancy complications (low- and high-risk). The inclusion criteria for pregnant women participated in this study were having an ability in the Persian listening and speaking to answer questions. The exclusion criteria were limited to the unwillingness to cooperate in the plan and the lack of access to patient information and medical adherence.

DATA COLLECTION AND PROCESSING

Some trained health workers and midwives were qualified to interview recruited pregnant women by filling out questionnaires and to obtain their different health-related information from datasets stored in affiliated hospitals and healthcare centers. It was assumed that all the data on history, examinations, laboratory tests, and conducted treatments were recorded in record files of mothers' health. If any medical and health information was not entered in the record book, it would be deemed not to have been delivered.

This study was conducted in three stages through observation and completion of a researcher-made checklist before and after the ANC. This checklist composed of four sections: (i) a checklist for categorizing the pregnancy risk in pregnant women (low and high levels) according to the national PCGs, (ii) a questionnaire for recording the socio-demographic data, (iii) a checklist for women's adherence to PCGs content, and (iv) a checklist for pregnancy (maternal and neonatal) outcomes.

The content validity of the developed checklist was assessed based on the viewpoints of 10 by members of the expert panel who had a specialty in obstetrics and gynecology, and midwifery care. The reliability of this checklist was also evaluated in the presence of 10% of the population. The high checklist's reliability ($\alpha = 0.91$) was also confirmed by calculating the internal consistency reliability coefficient "Cronbach's alpha".

At first, a set of socio-demographic (e.g., age, parity, education level, nationality, and occupational status), and midwifery (e.g., trimester at the first ANC, number of ANC visits, the timing of the first ANC visit, and previous pregnancy history and complications) characteristics were collected. The questions on the checklist were based on the requirements of the national country guide for obstetric services (CGOS) of Iran, which were consistent with the treatment instructions for the first visit of ANC. Accordingly, a series of responses were resulted concerning maternal (e.g., anemia, asymptomatic bacteriuria (ASB), intimate partner violence (IPV), gestational diabetes mellitus (GDM), tobacco and substance abuse, human immunodeficiency virus (HIV), and nutritional interventions) and fetal (e.g., symphysis-fundal height (SFH), antenatal cardiotocography, sonography, and screenings) assessments. The checklist was again completed in the second visit performed in the next 4-6 weeks. Finally, the information about the maternal and neonatal outcomes was collected using an electronic information system and phone calls to the mother after childbirth. Mothers were evaluated based on the first visit before the 14th week of their pregnancy, the number of ANC visits, medical tests, follow-up examinations, sonographies, screenings, and the use of food and drug supplements.

All maternal and neonatal complications during pregnancy, delivery, and postpartum periods were examined. Maternal complications included antepartum vaginal hemorrhage (APH), pregnancy-induced hypertension (PIH) and hypertensive complications (e.g., preeclampsia (PE), eclampsia (E), and hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome), anemia, infection, near miss, mother's admission in ICU, and delivery mode of cesarean section (CS). The assessed neonatal complications were preterm labor (PTL), low-birth-weight (LBW), small gestational age (SGA), intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR), respiratory distress syndrome (RDS), stillbirth, neonatal jaundice, NICU, and neonatal mortality.

The ANC adequacy was evaluated in two ways, including (i) the content adequacy by assessing the compliance to recommended routine care according to the ANC guidelines of Iran, and (ii) the effect of mothers' complete adherence to the

ANC guidelines on the risk of maternal and neonatal complications. The Kotelchuck index was used to determine the adequacy or quality of ANC during pregnancy (16). One score was assigned for each variable adhered to, while non-adherence was scored zero. Non-adherence to each of the mandatory variables was also classified as incomplete or inadequate adherence. Adherence was defined according to the criteria of following the instructions and recommendations at all levels of care, using prevention processes, self-care, and seeking diagnosis and treatment. Based on the prepared checklist, the compliance of 80% or more was considered as "full compliance", and the incomplete compliance was considered under 80% (17).

DATA ANALYSIS

All the data analyses were carried out using IBM SPSS Statistics for Windows version 24.0 (SPSS Inc., Chicago, IL, USA) at a significance level set at $p < 0.05$. Categorical variables of participants' socio-demographic were expressed as frequency and percentage. The Chi-square test was used to analyze the data related to the previous pregnancy histories and the possible relationship between adherence to the PCGs and patient properties. The nonparametric test of Mann-Whitney was applied for other variables that were not distributed normally. The results of the incidences of maternal and neonatal complications were reported in percentages. A multivariate logistic regression analysis was also performed to examine women's compliance with ANC health services, socio-demographic characteristics, and maternal and neonatal outcomes. Factors influencing the maternal and newborn complications

as well as the effect of complete adherence on pregnancy outcomes were reported as odds ratio (OR) with their 95% confidence intervals (CI). Variables such as trimester of first ANC visit, maternal age, parity, the previous pregnancy complication, and any antenatal complication where appropriate were also adjusted. The association between the content adequacy and pregnancy outcomes was assessed using a binary logistic regression in terms of a full model multivariate regression. Adjusted risk estimates were then obtained with the regression model that were fit for variables such as maternal age, maternal education, risk status, and content adequacy.

RESULTS

Table I shows some socio-demographic and obstetric information of subjects who participated in this study. Among the total of 604 qualified pregnant women with the mean age of 29.29 ± 5.40 years, 51.3% ($n = 310$) and 48.7% ($n = 294$) were categorized into low-risk and high-risk groups, respectively. Most participants had the 18-35-year age range (85%) and the high-school diploma educational level (43.9%). 4.63% of pregnant women were illiterate, whereas 23.84% of individuals had a secondary education level (**table I**). 95.36% ($n = 576$) and 28 (4.64%) of the participants were ethnic Iranian and Afghan women, respectively. Only 11.1% of the studied women had a history of previous pregnancy complications, while 13.1% of people were diagnosed to have gestational diabetes mellitus (GDM) at their first antenatal visit. Most participants experienced a cesarean delivery mode (41.56%).

Table I. Some socio-demographic and obstetric characteristics of the study participants.

Variable	Sub-variables	Frequency [n (%)]	Incomplete adherence [n (%)] ^a	Complete adherence [n (%)] ^a
Total sample	-	604	173 (28.6)	431 (71.4)
Mean age (years)	-	29.29 ± 5.6	26.98 ± 4.4	31.71 ± 5.7
Age (years)	< 18	6 (1.0)	0 (0.0)	6 (1.4)
	18-35	514 (85.10)	145 (83.81)	369 (85.6)
	> 35	84 (13.90)	28 (16.19)	56 (13.0)
Parity	0	216 (35.76)	44 (25.43)	172 (39.90)
	1-2	348 (57.62)	107 (61.85)	241 (55.91)
	3-4	36 (5.96)	18 (10.40)	18 (4.17)

	≥ 5	4 (0.66)	4 (2.31)	0 (0.0)
Education	No education	28 (4.63)	21 (12.14)	7 (1.62)
	Primary school	81 (13.41)	42 (24.28)	39 (9.48)
	Secondary school	144 (23.84)	42 (24.28)	102 (23.66)
	Diploma	265 (43.87)	56 (32.37)	209 (48.49)
	University	86 (14.24)	12 (6.93)	74 (17.17)
Nationality	Iranian	576 (95.36)	151 (87.28)	425 (98.60)
	Afghan	28 (4.64)	22 (12.72)	6 (1.40)
Occupational status	Employed	584 (96.69)	167 (96.53)	417 (96.75)
	Unemployed	20 (3.31)	6 (3.47)	14 (3.25)
Trimester at the first ANC	First	556 (92.05)	137 (79.20)	419 (97.22)
	Second	48 (7.95)	36 (20.80)	12 (2.78)
Previous delivery ^b	Yes-CS	251 (41.56)	69 (39.88)	182 (42.23)
	Yes-NVD	120 (19.87)	51 (29.48)	69 (16.01)
	Yes-CS/NVD	172 (28.47)	9 (5.20)	8 (1.85)
	No	61 (10.1)	44 (25.43)	172 (39.91)
Previous pregnancy complication	Yes	67 (11.1)	26 (15.03)	41 (9.51)
	No	537 (88.9)	147 (84.97)	390 (90.49)

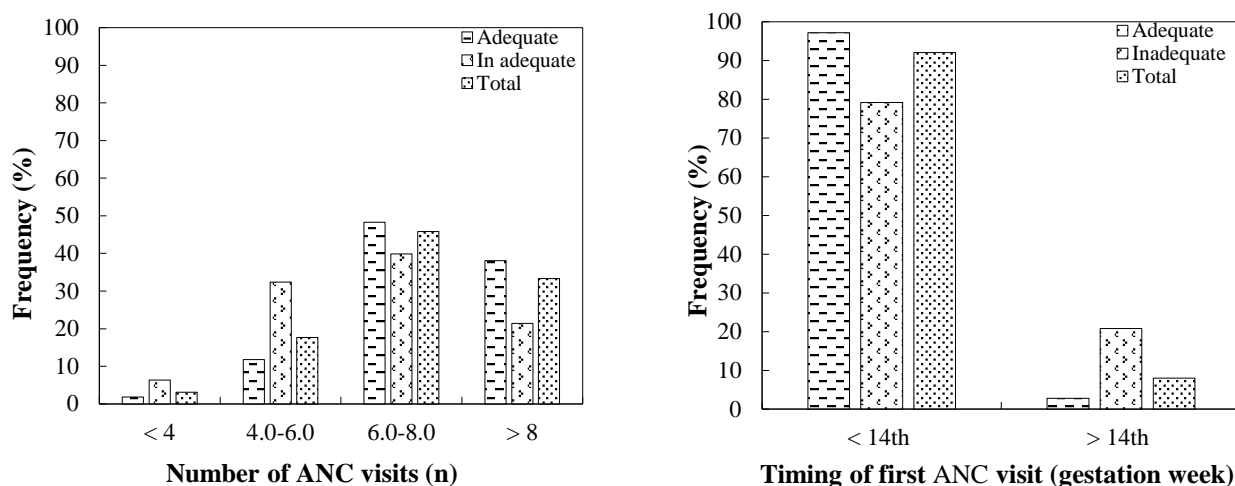
^a Incomplete and complete adherence were equal to < 80% and ≥80%, respectively.

^b CS and NVD are cesarean section and natural vaginal delivery, respectively.

According to the results of the Kotelchuck adequacy of prenatal care index (figure 1), 92.05% (n = 556) of women generally had their first antenatal visit during the first trimester (< 14 weeks). Besides, 96.85% of them attended at ANC clinic

over four times visit during their pregnancy. Also, 71.36% (n = 431) and 28.64% (n = 173) of women had complete (≥ 80%) and incomplete (< 80%) adherence to the PCGs, respectively (figure 1).

Figure 1. Percent distribution of the number of ANC visits (a), and of the timing of first ANC visit (b) in the population groups with inadequate (n = 173, 28.6%) and adequate (n = 431, 71.4%) adherence to PCGs.



The risk incidence rate of maternal and neonatal complications is given in **table II**. Although no maternal mortality was found amongst the

study participants, the risk of maternal near miss was assessed to be 10.43%. The risk rate for any pregnancy, delivery, or post-partum

complication type was 32.8%. The incidence of PIH and hypertensive complications (e.g., PE, E, and HELLP syndrome) and antepartum vaginal bleeding were 4.63 and 1.32%, respectively. The incidence rate of mother's admission in ICU, pregnancy anemia, infection after delivery, and cesarean section delivery were 9.93, 2.65, 3.80, and 70.53%, respectively (**table II**).

The incidence rate of PTLs, LBW babies, stillbirths, and neonatal mortalities were 15.56, 9.60, 0.66, and 1.32%, respectively. Moreover, the risk incidence of SGA, and neonatal RDS or asphyxia/difficulty in breathing, and admissions of infants in NICU were 1.49, 9.43, and 21.02%, respectively. The risk of other neonatal complications such as IUFD and IUGR were 0.66 and 1.65%, respectively (**table II**).

Table II. The incidence risk rate of maternal and neonatal complications and their percent distribution in the population groups with inadequate and adequate adherence to PCGs.

Sub-group	Complication ^a	Incidence [n (%)]	Incomplete adherence [n (%)] ^b	Complete adherence [n (%)] ^b	p-value
Maternal	APH	8 (1.32)	4 (2.31)	4 (0.93)	> 0.05
	PIH (e.g., PE, E, HELLP)	28 (4.63)	8 (4.62)	20 (4.64)	0.427
	Anemia in pregnancy	16 (2.65)	5 (2.89)	11 (2.55)	> 0.05
	Infection	23 (3.80)	4 (2.31)	19 (4.41)	0.373
	Delivery by CS	426 (70.53)	112 (64.74)	314 (72.85)	0.008
	Near miss	63 (10.43)	17 (9.82)	46 (10.67)	0.424
	ICU	60 (9.93)	15 (8.67)	45 (10.44)	0.127
Neonatal	PTL	94 (15.56)	30 (17.34)	63 (14.61)	0.402
	LBW	58 (9.60)	15 (8.67)	43 (9.97)	0.213
	SGA	9 (1.49)	3 (1.73)	6 (1.39)	0.476
	IUFD	4 (0.66)	1 (0.57)	3 (0.69)	0.556
	RDS	57 (9.43)	30 (17.34)	27 (6.26)	0.012
	Stillbirth	4 (0.66)	3 (1.73)	1 (0.23)	> 0.05
	IUGR	10 (1.65)	5 (2.89)	5 (1.16)	0.158
	NICU	127 (21.02)	47 (27.16)	80 (18.56)	0.01
	Mortality	8 (1.32)	4 (2.31)	4 (0.93)	> 0.05

^a APH: antepartum hemorrhage, PIH: pregnancy-induced hypertension, PE: preeclampsia, E: eclampsia, PIH: HELLP: hemolysis, elevated liver enzymes, and low platelets (syndrome), CS: cesarean section, ICU: intensive care unit, NICU: neonatal intensive care unit, PTL: preterm labor, LBW: low-birth-weight, SGA: small gestational age, IUFD: intrauterine fetal death, IUGR: intrauterine growth restriction, and RDS: respiratory distress syndrome.

^b Incomplete (n = 173) and complete (n = 431) adherence were equal to < 80% and ≥80%, respectively.

The logistic regression results showed that there was a significant association between women's adherence to the PCGs and their education level (**table III**). Women with higher education were more adherent (OR = 10.718, 95% CI (3.086-37.223), p = 0.0001) and the employed women compared to housewives had lower prenatal care compliance (OR = 0.273, 95% CI (0.083-0.910), p = 0.03). Also, Afghan women compared to Iranian women exhibited lower prenatal care adherence (PCA) (OR = 0.129, 95% CI (0.042-0.397), p = 0.0001). Women with GDM also had lower PCA (OR = 0.323, 95% CI (0.196-0.534), p = 0.0001). There was a significant negative association between PCA with parity so that

women's PCA decreased with an increase in parity (OR = 0.011, 95% CI (0.001-0.221), p = 0.003) (**table III**). However, no significant differences were found for the maternal outcomes related to PCA (**table III**).

A significant correlation was detected between women's PCA and a number of neonatal outcomes, including: women with lower PCA were more at risk of RDS (OR = 0.273, 95% CI (0.121-0.617), p = 0.012), as well as babies admitted to NICU (OR = 0.593, 95% CI (0.298-1.180), p = 0.01).

In the multivariable analysis, no significant difference was obtained for PCA related to LBW, SGA, and IUGR (p > 0.05) (**table IV**).

However, there was a significant association between women's PCA, the pregnancy outcomes and the beginning of the visit in the first trimester (OR = 0.178, 95% CI (0.084-0.376), p = 0.0001).

Table III. The logistic regression analysis of complete adherence to PCGS and socio-demographic/obstetric data before and after adjusting the confounding factors.

Variable	Sub-variables	Unadjusted		Adjusted	
		OR(%95 CI)	p-value	OR(%95 CI)	p-value
Age (year)	< 18	1		1	
	18-35	1.436(0.710-2.904)	0.314	1.374 (0.676-2.793)	0.380
	> 35	1.103(0.489-2.485)	0.814	1.036 (0.456-2.353)	0.933
Education	No education	1		1	
	Primary school	2.786 (1.067-7.276)	0.036	1.715 (0.558-5.271)	0.234
	Secondary school	7.286 (2.881-18.425)	0.0001	4.183 (1.402-12.482)	0.003
	Diploma	11.196 (4.530-27.672)	0.0001	5.948 (2.033-17.398)	0.0001
	University	18.500 (6.470-52.898)	0.0001	10.718 (3.086-37.223)	0.0001
Occupational status	Housewife	1		1	
	Employed	0.934 (0.353-2.473)		0.273(0.083-0.910)	0.033
Nationality	Iranian	1		1	
	Afghan	0.097(0.039-0.244)	0.0001	0.129(0.042-0.397)	0.0001
Abortion	No	1		1	
	Yes	0.769(0.525-1.129)		1.156(0.637-2.097)	0.633
Gestation diabetes	No	1		1	
	Yes	0.351(0.217-0.570)	0.0001	0.323(0.196-0.534)	0.0001
Gravid history	1	1		1	
	2-4	0.448(0.284-0.707)	0.001	0.351(0.133-0.978)	0.035
	≥ 5	0.021(0.003-0.167)	0.0001	0.011(0.001-0.221)	0.003
Healthcare center type for the first ANC visit	Private clinic	1		1	
	Public hospital	0.386(0.208-0.715)		0.394(0.209-0.743)	0.004

Table IV. The effect of complete adherence to PCGs on pregnancy outcomes using the logistic regression analysis before and after adjusting the confounding factors.

Variable ^a	Sub-variables	Unadjusted		Adjusted	
		OR(%95 CI)	p-value	OR(%95 CI)	p-value
Trimester at the first ANC	First	1		1	
	Second	0.109(0.055-0.215)	0.0001	0.178(0.084-0.376)	0.0001
Delivery age	> 37 weeks	1		1	
	< 37 weeks	1.225(0.762-1.972)	0.402	1.334(0.752-2.368)	0.325
Birth weight (g)	< 2500	1		1	
	2500-4000	0.849(0.458-1.574)	0.604	0.621(0.294-1.313)	0.213
	>4000	1.134(0.320-4.019)	0.846	0.796(0.206-3.066)	0.740
ICU	No	1		1	
	Yes	1.228(0.665-2.267)	0.511	2.126(0.807-5.604)	0.127
NICU	No	1		1	
	Yes	0.611(0.404-0.924)	0.020	0.593(0.298-1.180)	0.010
Near miss	No	1		1	

	Yes	1.098 (0.610-1.971)	0.758		1.295(0.687-2.440)	0.424
Infection	No	1			1	
	Yes	1.750(0.542-5.649)	0.232		1.657(0.545-5.037)	0.373
IUGR	No	1			1	
	Yes	0.394(0.113-1.380)	0.145		0.339(0.083-1.380)	0.158
IUFD	No	1			1	
	Yes	1.252(0.129-12.124)	0.672		0.469(0.038-5.819)	0.556
RDS	No	1			1	
	Yes	0.319(0.183-0.554)	0.0001		0.273(0.121-0.617)	0.012
SGA	No	1			1	
	Yes	0.800(0.198-3.235)	0.754		0.562(0.115-2.744)	0.476
Jaundice	No	1			1	
	Yes	1.826(0.829-4.020)	0.110		2.664(1.063-6.681)	0.030
PE	No	1			1	
	Yes	1.004(0.433-2.324)	0.993		0.616(0.187-2.034)	0.427
Previous delivery	CS ^b	1			1	
	NVD ^b	0.555(0.355-0.869)	0.010		0.539(0.342-0.848)	0.008

^a ICU: intensive care unit, NICU: neonatal intensive care unit, IUFD: intrauterine fetal death, IUGR: intrauterine growth restriction, RDS: respiratory distress syndrome, SGA: small gestational age, and PE: preeclampsia.

^b CS: cesarean section, and NVD: natural vaginal delivery.

DISCUSSION

This study aimed to evaluate the relationship between the compliance of pregnant women with the content of the standard guidelines for prenatal care and the improvement of maternal and neonatal outcomes. 51.3% of pregnant women in this study were classified into the low-risk group, while 71.4% of the total population had complete adherence to the first ANC guidelines. This fact shows a better assessment than the one found in Yeoh et al. (11) who reported 49.8% of the full adherence level. Moreover, they found Malaysian pregnant women at lower risk (77%) showed higher adherence to ANC guidelines (11). Results showed that there was no significant correlation between the participants' age and adherence degree. No meaningful relationship between these two variables in our study was because of the similarity of risk level in the different age groups. However, Amoakoh-Coleman et al. (18) reported a significant relationship between Ghanaian pregnant women and the adherence level to PCGs so that the adherence level of pregnant women to this care program was increased as their age increased. It seems that older women had higher adherence to the ANC guideline due to their awareness of the important role

in improving maternal and neonatal outcomes. In contrast, Yaya et al. (6) realized that older women had less care and less compliance with the ANC guideline. This fact may be ascribed to the high number of study participants in the age group above 35 years, so that this relationship was significant, and women in this group were less adherent. However, better mothers' education is required to complete adherence to the guidelines for providing more exact information to the stakeholders about the content of the instructions and how they might be helpful to improve the delivery of their services. Thus, women with lower education levels significantly had lower adherence to prenatal care during their pregnancy. Similar results have already been reported from other related studies (2,11,19). Agha and Tappis (20) also showed that the lowest compliance level was related to illiterate women owing to their lower awareness of receiving ANC guidelines. The current study also showed that women with higher parity had lower adherence to PCGs than other women, which was in line with the findings reported from other studies (6,11,18). Our study showed that employed women were less adherent to PCGs. This finding could be due to the lack of time for working women to go to public health

clinics or their referral to private settings. We found that the pregnancy number had a significant effect on the mother's adherence to ANC guidelines so that the compliance rate was reduced with an increase in childbirth numbers. It seems that caring for smaller children is a major problem for most pregnant women, requiring a child nurse to care for them, while nulliparous women do not have such worry and possess enough time to refer to healthcare centers. A similar result was obtained by Yaya et al. (6) who reported that nulliparous women compared to multiparous ones were more adherent to PCGs. Conversely, Amoakoh-Coleman et al. (18) mentioned higher compliance with PCGs by multiparous women. They explained that these women were more aware of the importance of these maternal and neonatal care and outcomes. The results showed that pregnant women with history of CS delivery were more adherent than women with natural vaginal delivery (NVD). Lower safety of CS delivery for pregnant women compared to NVD along with the requirement to receive more care and follow-up due to pre-operative hospitalization might result in the more need for health cares and compliance with PCGs. A similar result was reported by Milcent and Zbiri (21) who French pregnant women with a history of CS delivery received more care with more clinical tests and sonography scans.

Also, there was no significant relationship between women's adherence to the antenatal guideline and PTL, ICU, near miss, infection in postpartum, preeclampsia, postpartum and hemorrhage complications. Although some studies have demonstrated that complete adherence to the first antenatal guideline resulted in reduced risk of delivery and neonatal complications (7,11,18). This fact may be due to receiving adequate prenatal care in these groups. Moreover, pregnant women (mainly, with a history of GDM and PE) in most Iranian medical centers will be stayed in the ICU for up to 24 hours postpartum to prevent any maternal complication and then admitted to the ward after being assured of a stable physical condition. Amoakoh-Coleman et al. [18] also reported the same finding and attributed it to the therapeutic methods used in healthcare centers. However, Yeoh et al. (7) pointed out that there was a significant association between adherence of women to the

content of PCGs and pregnancy outcomes so that women with higher adherence had a reduced rate of PTL and maternal and neonatal complications. In the present study, the results showed that women who had their first ANC visit before the 14th week of pregnancy exhibited a better maternal outcome and a significant relationship with women who initially visited for ANC after the 14th week of pregnancy.

Pregnant women with the first ANC visit after the 14th week have lost most of the care they need, such as targeted tests, screening, and sonography during pregnancy. Therefore, there was not enough time for health care providers to prevent or treat these complications. Similar findings were obtained by other researchers (7,18,20).

Our results revealed that the risk of RDS and NICU could be increased when there was a low adherence to the prenatal guideline. Other similar studies have also reported an increase in RDS and babies admitted to NICU under lower adherence to PCGs (7).

We found that there was no significant between women's adherence to the ANC guideline with LBW, stillbirth, and preterm birth.

However, some studies demonstrated the significant complete adherence to the first antenatal guideline resulted in reduced risk of LBW, stillbirth, and preterm birth (11, 21-25). The current study indicated the significant role of the quality of prenatal care in evaluating the desired outcomes. The complete adherence to ANC guidelines resulted in a reduced risk of maternal and neonatal complications, as earlier evidenced by other researchers (4).

STUDY LIMITATIONS

In this study, the 3rd level hospitals in Tehran were chosen as research sites, because more than 92% of the pregnant women were referred to these hospitals, and only 8% of them visited private hospitals, where prenatal care was performed differently. Furthermore, pregnant women referred to these hospitals were not available to the researchers. We also used researcher-made instruments to achieve our study objectives, which required validity and reliability analyses.

CONCLUSION

Results of the present study revealed the significant effect of adherence to ANC guidelines on neonatal conditions, while no substantial impact was obtained on maternal outcomes. Therefore, implementing the programs promoting complete adherence to the ANC guideline will be necessary to improve the outcomes of neonatal service deliveries.

However, the early ANC should be encouraged amongst the population. Further investigations on the quantity and quality of prenatal care are

suggested to enhance the effectiveness of prenatal care for high-risk pregnancies. There is also a need to explore and understand the possibility of expository mechanisms for these observations. On the other hand, the assessment of ANC content in this survey was based on the current national ANC guidelines. As the Iranian guidelines compared to the evidence-based ones from other countries (especially, in Australia and the UK) resulted in better maternal and neonatal health outcomes, this program might be used as an appropriate practical pattern in the maternity healthcare setting in other countries.

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In vitro fertilization and psychological stress: new insight about different routes of progesterone administration

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ABSTRACT

Infertility treatment is a stressful process and factors like anxiety and preoccupation could affect the success of In Vitro Fertilization (IVF) or other assisted reproductive techniques. Moreover, luteal phase support (LPS) in IVF cycles is recommended. Our aim was to analyze the effects of LPS with intramuscular and subcutaneous progesterone on stress hormones (cortisol and prolactin). We analyzed one hundred-thirty women undergoing their first IVF cycle and then randomized in two groups: group A (65 patients) received 33 mg/day of intramuscular in oil-progesterone from pick-up and 50mg/day from embryo transfer, group B (65 patients), instead, received 25 mg of subcutaneous water soluble-progesterone from pick-up. Cortisol and prolactin serum levels were obtained at day+7 from oocyte retrieval. Our results showed that the values of prolactin and cortisol were statistically significantly higher in group A compared to the group B. Subcutaneous progesterone treatment, in fact, is associated with lower cortisol and prolactin levels, suggesting new therapeutic opportunities in IVF cycles to reduce patients' distress and improve quality of life.

SOMMARIO

Il trattamento dell'infertilità è un processo stressante e fattori come l'ansia e la preoccupazione potrebbero influenzare il successo della fecondazione in vitro (IVF) o di altre tecniche di riproduzione assistita. Inoltre, il supporto alla fase luteale (LPS) è raccomandato nei cicli di IVF. Il nostro obiettivo è stato quello di analizzare gli effetti sugli ormoni dello stress (cortisolo e prolattina), della LPS con progesterone intramuscolare e sottocutaneo. Abbiamo analizzato centotrenta donne sottoposte al primo ciclo di IVF e le abbiamo randomizzate in due gruppi: il gruppo A (65 pazienti) ha ricevuto 33 mg/die di progesterone intramuscolare dal giorno del pick-up e 50 mg/die dal giorno del trasferimento dell'embrione, il gruppo B (65 pazienti), invece, ha ricevuto 25 mg di progesterone, solubile in acqua, per via sottocutanea dal giorno del pick-up. I livelli sierici di cortisolo e prolattina sono stati valutati dopo sette giorni dal pick-up. I nostri risultati hanno mostrato che i valori di prolattina e cortisolo erano statisticamente più alti nel gruppo A rispetto al gruppo B. Il trattamento sottocutaneo di progesterone, infatti, è associato a livelli più bassi di cortisolo e prolattina, suggerendo nuove opportunità terapeutiche nei cicli di IVF per ridurre lo stress dei pazienti e migliorare la qualità della vita.

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Key words: IVF; cortisol; prolactin; luteal phase support; pregnancy; progesterone

INTRODUCTION

The diagnosis of infertility is a stressful event for the couple and may cause negative symptoms such as depression, anxiety and psychosomatic symptoms, potentially interfering with the medical procedures. Different types of psychological stressors in humans are related to an increased serum levels of the anterior pituitary hormone prolactin (PRL) and to an activation of the hypothalamic-pituitary-adrenal (HPA) axis with the synthesis and release of cortisol (CORT) by the adrenal cortex (1). Such adaptive mechanisms allow the body to maintain physiological stability. Therefore, PRL and CORT can be considered stress biomarkers. In vitro fertilization (IVF) represents the treatment of choice for over 1.000.000 infertile couples each year (2), and among infertility treatment it is the most stressful and emotionally demanding procedure because of daily injections, blood draws, ultrasound checks, oocyte retrieval, and the possible failure of the cycle (3,4,5). The role of stress in IVF has long been a topic of interest. Questions include whether the process of fertility treatment is stressful, whether stress or anxiety has an impact on success of fertility treatment, and whether interventions to decrease stress are useful (6,7). The perception that psychological stress may prevent a woman from attaining and maintaining a pregnancy has become widely accepted (8-12). Besides, other authors suggest that couples entering an IVF-treatment program are usually psychologically well-adjusted (3). Moreover, women's anxiety and general distress change during the course of one treatment cycle: a pioneering study (8) monitored the entire course of the IVF treatment cycle by daily record of subjective general distress and reported a significant increased distress at the end of the cycle, between oocyte retrieval and pregnancy test. Such data were confirmed by other studies (8, 13-16). IVF cycles are associated with abnormal luteal phase leading to poor endometrial development and asynchrony of endometrial receptivity, therefore a pharmacological support is recommended (17,18). Progesterone for luteal phase support (LPS) is available for intramuscular (IM) injection in oil, for vaginal preparation, either in oil-capsule or vaginal gel, and for oral capsule. Recently, a water-soluble injectable progesterone has become available for subcutaneous (SC)

injection. The purpose of this study was to investigate the effects of LPS with IM and SC progesterone on the stress hormones (CORT and PRL) that are known to influence reproductive outcomes in IVF cycles.

MATERIALS AND METHODS

This was a prospective study of women undergoing their first IVF treatment for primary infertility, between March 2017-October 2017, to the Department of Obstetrics and Gynecology of University of Campania "Luigi Vanvitelli" in Naples (Italy). All patients signed informed consent form to be part of a study. The experimental study was conducted in accordance with principles of the Helsinki Declaration of 1975, using routine clinical practice procedures usually performed during IVF cycles; such procedures did not involve additional risks to the patients and all the medical decisions concerning individual patients were not affected by the study. The study was approved by Local Review Board. All the women were < 37 years old, showed regular menstrual cycles, body mass index (BMI) between 20 and 25 kg/m², serum hormonal profile within the normal range (FSH and LH <10 IU/ml, E₂ <50 pg/ml, prolactin <30 ng/ml), normal karyotype, normal uterine cavity as diagnosed at ultrasonographic and hysteroscopy examination. Exclusion criteria were: acute illness, chronic disorders, PCOs, endometriosis, organic dysfunction. Patients included in the study underwent ovarian stimulation with our standard protocol. Briefly, all the patients underwent a standard down-regulation with GnRH analogue hormone at a dose of 0.1mg/day (Triptoreline, Decapeptyl, Ipsen, Milan, Italy) until estradiol levels ≥ 40 ng/mL and no follicle >7 mm; patients over 35 years old received a sequential stimulation protocol starting with uFSH (Fostimon, IBSA, Switzerland) for 6 days according to a step-down approach (225IU for 4 days and 150 IU for the last two days) and then shifting to rFSH at the standard dosage of 150 IU; patients under 35 years old received a standard protocol with rFSH (Gonal- F; Serono, Rome, Italy), at a daily dose of 225 IU for 4 days and 150IU for the last two days. By the seventh day therapy was personalized according to the hormonal and ultrasonographic assessment.

Ovulation induction was monitored by vaginal ultrasound and hormonal assessment every second-third day. When at least three follicles had reached a diameter of 18 mm, a single SC bolus of 10.000 IU of hCG (Gonasi HP 10000; IBSA, Rome, Italy) was administered. Transvaginal follicular aspiration was performed 34-36h after hCG administration. Oocytes retrieved were cultured in Petri dishes in IVF HTF Buffer (Cook Medical) at 37°C in a humidified 5% carbon dioxide/95% air environment. The oocytes were denuded enzymatically using 80 IU/ml hyaluronidase (Sage) and mechanically. The semen was processed with 90% - 45% discontinuous Gradient (SAGE In-Vitro Fertilization) centrifugation at 1700 g for 12 minutes. After IVF, the resulting embryos were cultured in IVF Cleavage Medium (Cook Medical) at 37° C under 5% carbon dioxide in air environment until day+3. At day+4, we changed the culture media and used IVF Blastocyst Medium (Cook Medical) at 37° C under 5% carbon dioxide in air environment until the day of embryo transfer. Serum levels of hCG were measured 14 days after ET and, if positive, were obtained every 3-6 days until an intrauterine gestational sac was demonstrated by US examination. Until the pregnancy test, patients were invited to follow their usual lifestyle, workers were invited to continue their occupation. Patients were randomized in two groups according to the luteal phase support: group A (65 patients) received 33 mg/day of IM in oil-progesterone starting from pick-up day and then 50mg/day from embryo transfer (ET) day, group B (65 patients) received 25mg of SC water soluble-progesterone from pick-up day. Blood samples for PRL e CORT dosage were obtained from all subjects at day +7 from oocyte retrieval by venipuncture in the morning between 7.30 and 8.30 and stored until analysis. PRL e CORT serum levels are very susceptible to multiple variables, like the time of blood collection, fasting state, protocol of stimulation. To reduce possibly bias, all patients had serum levels checked before 8.30 A.M. and were not fasting; all patients received low-dose aspirin by ET day. Blood serum levels for CORT and PRL detection were by Immunolite from DPC/Siemens (Princeton, NJ) (PRL intra-assay coefficient of variation [CV] 1/4 6.8%; interassay CV 1/4 9.6%; CORT intra-assay CV 1/4 8.8%; inter-assay CV 1/4 10%). Data are shown as mean ± standard

deviation (SD). For statistical analysis, t-test, χ^2 , log rank analysis, and analysis of variance were used as appropriate. The results were statistically significant when p-value was <0.05.

RESULTS

One-hundred thirty patients were enrolled for a prospective study. All patients completed IVF stimulation, egg retrieval, ET and performed a pregnancy test. Group A and B were similar concerning baseline characteristics and stimulation data (**table I**).

Table I. Baseline characteristics and stimulation data in two group.

	Group A (n=65)	Group B (n= 65)	p-value
Age (years)	33.2 ± 2.8	33.9±3.1	ns
Body mass index (kg/m ²)	23.3. ± 2.3	24.2±2.0	ns
Time of infertility (years)	1.9 ± 1	2.2 ± 0.5	ns
Day-3 FSH (IU/mL)	7.4 ± 1.9	6.9 ± 2.3	ns
AMH (ng/mL)	2.3 ± 1.1	2.8 ±1.8	ns
Total dose of FSH used (IU)	2.098.9±837.6	2.122.5±786.5	ns
Estradiol on hCG day (pg/mL)	1.872±684.6	1.928.2±708.1	ns

Abbreviations: ns, not significant

All patients had a regular lifestyle during LPS, reporting neither bleeding or spotting, nor any health problem. No statistically significant difference in number of oocyte retrieval,

fertilization rate, number of transferred embryo and pregnancy rate were found among two group. The data were show in **table II**.

Table II - Data from IVF cycles.

	Group A (n=65)	Group B (n= 65)	p-value
Retrieved oocytes (n)	9.7 ± 4.5	10.2 ± 3.9	ns
Metaphase II oocytes (n)	6.5 ± 1.7	6.7 ± 1.6	ns
Fertilization rate (%)	85.7	83.8	ns
Transferred embryo (n)	2.6 ± 0.3	2.2 ± 0.5	ns
Pregnancy (n)	8	9	ns
Pregnancy rate (%)	24.2	25.7	ns

Abbreviations: ns, not significant

The values of PRL and CORT were statistically significantly higher in group treated by IM route compared to the group treated by SC route (27.5 ± 3.3 ng/ml vs 21.8 ± 3.3 ng/ml, 345.5 ± 29.9 µg/L vs 310.5 ± 28.7 µg/L) (**table III**).

Table III - Values of Cortisol (COR) and Prolactin (PRL).

	Group A (n=65)	Group B (n= 65)	p-value
COR (µg/L)	345.5 ± 29.9	310.5 ± 28.7	<0.05
PRL (ng/ml)	27.5 ± 3.3	21.8 ± 3.3	<0.05

DISCUSSION

Many investigators discussed the role of stress on reproductive failure (19,20,21). It is well demonstrated that the infertility and IVF are conditions of chronic stress, which is highlighted

in the final stages of the cycle when the expectation becomes more intensive (22). Moreover, stress can favor Human Papillomavirus persistence, a sexually transmitted infection, that may affect fertility and alter the efficacy of assisted reproductive technologies (23-26). Many reports show that stress levels across stages of the IVF cycle vary between pregnant and non-pregnant women (8,13-16,27). Perceived stress questionnaires are the most widely used psychological instrument for measuring the subjective perception of stress, but they may result unreliable: it is well known that infertile patients suppress their feelings of stress because they want to show the clinic that they are functioning well both socially and psychologically (28-30).

PRL and CORT may appropriately be used as objective indicators of stress levels.

Our data showed that treatment with SC progesterone is associated with statistically significant lower levels of CORT and PRL during the LPS of an IVF cycle. It can be speculated that patients showed higher compliance to SC route while are bothered by progesterone IM injection.

Since the SC route is a friendly administration already daily experienced by the patients during ovulation induction with gonadotropins, switching progesterone supplementation from IM to SC administration could modify the levels of stress markers (31-33). Moreover, IM progesterone administration may be burdened by local inflammation and pain and increased the drop-out rate from the treatment, whereas SC route is less painful and is associate with a handily daily management of LPS (12,34).

The patient should not manage anxiety related to a new type of administration and may self-manage therapy remaining independent from another person. Moreover, SC administration allows the woman to face each injection with greater confidence and concern and to reduce the possibility of drop out at any stage of the present and possible future cycle.

In conclusion, a friendly route of administration may lead primarily to reduce levels of stress indicators such as PRL and CORT, then to improve patients' quality of life, to optimize therapeutic strategy as regards the IVF outcome, finally to reduce dropout rate from IVF program.

Our results suggest a new opportunity in IVF therapy to reduce patients distress and improve

the quality of life, supporting the development of new research perspectives and new therapeutic strategy for the management of LPS during IVF.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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The role of serum potassium and sodium levels in the development of postpartum hemorrhage. A retrospective study

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ABSTRACT

Objectives. Primary postpartum hemorrhage (PPH) represents the first responsible cause of maternal mortality, leading at least a quarter of deaths during childbirth and in the puerperium. Most cases of PPH occur in women who do not have distinct risk factors. Voltage-gated ions channels, particularly the potassium channels, play an essential role in contractility regulation of the uterus. The aim of our study was to identify the possible role of the pre-delivery serum sodium (Na⁺), potassium (K⁺) electrolytes levels and Na⁺K product in PPH.

Methods. We conducted a single-institution retrospective study on all patients consecutively referred to our institution for spontaneous delivery between 1 January 2011 and 31 December 2018. Among these, patients with PPH were compared to controls patients selected according to specific inclusion-exclusion criteria. All information regarding patient's characteristics were retrospectively collected, particularly, pre-delivery blood tests, including serum electrolytes (such as K⁺ and Na⁺) were recorded and compared

between cases and controls by Chi-square and U Mann-Whitney tests, as appropriate. Logistic regression analysis was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of PPH associated with Na⁺, K⁺ and Na⁺K serum levels. In the analysis a crude (simple) and an adjusted model (adjusted for episiotomy and perineal laceration) were used. To evaluate the best PPH predictive score, a ROC curve (Receiver Operating Characteristic) was used.

Results. A lower statistically significant serum level of K⁺ and Na⁺K product were recorded among the primary PPH cases compared to the control group (p<0.001). The adjusted ORs (95%CI) for K⁺ and the product Na⁺K were 19.40 (95% CI 2.40-156) and 8.78 (95% CI 1.96-39), respectively.

Conclusions. A level of electrolytes, particularly for K⁺ serum level, lower than the non-pregnancy threshold/median, could be considered as a possible pre-delivery risk factor for primary PPH.

SOMMARIO

Obiettivi. L'emorragia primaria del postpartum (PPH) rappresenta la prima causa di mortalità materna, provocando almeno un quarto dei decessi durante il parto e il puerperio. La maggior parte dei casi di PPH primaria si verifica in donne che non presentano fattori di rischio evidenti. I canali degli ioni dipendenti dalla tensione, in particolare i canali del potassio, svolgono un ruolo essenziale nella regolazione della contrattilità dell'utero. Lo scopo del nostro studio era di identificare il possibile ruolo del valore sierico pre-parto di elettroliti quali sodio (Na⁺), potassio (K⁺) e del loro prodotto (Na*K) nella PPH primaria.

Metodi. Abbiamo condotto uno studio retrospettivo su tutti i pazienti afferiti consecutivamente al nostro Centro per l'espletamento del parto spontaneo, tra il 1° gennaio 2011 e il 31 Dicembre 2018. Tra questi, i pazienti con PPH primaria sono stati confrontati con i controlli selezionati in base a specifici criteri di inclusione-esclusione. Tutte le informazioni relative alle caratteristiche delle pazienti sono state raccolte in modo retrospettivo (tramite le cartelle clini-

che), in particolare sono stati presi in considerazione gli esami del sangue pre-parto, compresi gli elettroliti sierici (come K⁺ e Na⁺). Quindi, i due gruppi sono stati confrontati mediante i test Chi-quadrato e U Mann-Whitney.

Il rischio di PPH in relazione ai livelli sierici di Na⁺, K⁺ e Na*K è stato stimato utilizzando modelli di regressione logistica grezzi e multivariati. Infine, per stimare il cut off, degli elettroliti indagati, predittivo di PPH è stata utilizzata la curva ROC (Receiver Operating Characteristic).

Risultati. Sono stati riscontrati valori sierici di K⁺ e del prodotto Na*K statisticamente più bassi nel gruppo con PPH primaria rispetto al gruppo di controllo ($p < 0.001$).

Gli ORadj per il K⁺ e per il prodotto Na*K erano rispettivamente 19,4 (95% IC 2.40-156) e 8,78 (95% IC 1.96-39).

Conclusioni. I livelli sierici di elettroliti, in particolare quello del K⁺, al di sotto del valore soglia/mediana, potrebbe essere considerato un possibile fattore di rischio pre-parto per la PPH primaria.

INTRODUCTION

Primary postpartum hemorrhage (PPH) is defined, by the World Health Organization (WHO), as blood loss greater or equal to 500 mL and 1000 mL, within the 24 hours, after vaginal delivery and cesarean section, respectively (1-7). Postpartum hemorrhage has a worldwide prevalence of 3-5% and is the first cause of maternal mortality, especially in low-income countries, being responsible for at least a quarter of deaths during childbirth and puerperium (8-13). Most cases of PPH occur in women who do not have distinct risk factors. The most common cause is uterine atony (about 70% of cases), followed by perineal and vaginal tears and/or trauma (20%), placenta tissue retention, placental abnormalities (9%), and coagulopathies (1%) (6,8,11,14,15). In clinical practice, they are summarized as the "4Ts": tone, tissue, trauma and thrombin (2,10,12,16). Complications of postpartum hemorrhage are: anemia, blood transfusion, dilutional coagulopathy, Sheehan syndrome or postpartum pituitary necrosis, fatigue, myocardial ischemia, orthostatic hypotension, postpartum depression, and death (5,16-20). However, the molecular mechanisms behind the most common cause of primary PPH are not well understood. A fundamental role is played by oxytocin that acts not only as a ligand for specific membrane receptors but also as a ligand of calcium channels, modulating both the myosin light chain kinase (MLCK), the myosin phosphatase, and the release of inositol trisphosphate (IP₃). The outcome of these mechanisms is the increase in Ca⁺⁺ level, which triggers uterine contraction (21-23). Calcium also depolarizes the plasma membrane of the smooth uterine cells, allowing the activation of voltage-gated potassium and sodium channels (23,24). During pregnancy, the uterus is quiescent and non-responsive, and it has a hyperpolarized membrane potential that is close to that of potassium (25,26). Conversely, at the end of pregnancy and during the labor, myocellular depolarization occurs with the development of uterine contractions (26). There is a common agreement that voltage-gated ions channels, particularly the potassium one, play an essential role in the regulation of the membrane potential and, therefore, in the contractility of the human uterus (27). Specifically, the Kir 7.1 potassium channel has demonstrated to play

a critical role in regulating uterine excitability during pregnancy and might be involved in some PPH cases (28,29). Studies have long focused on the role of potassium channels in uterine contractility, but it is not clear at all the relationship with PPH (26,30-32). The aim of our study was to identify the possible role of the pre-delivery levels of sodium (Na⁺) and potassium (K⁺) serum electrolytes in the PPH development.

MATERIALS AND METHODS

This is a retrospective study of pregnant women reported with primary PPH and compared with healthy controls. The study was conducted according to STROBE (Strengthening the reporting of observational studies in epidemiology guidelines (33).

POPULATION SELECTION CRITERIA

After obtaining approval by the Institutional Review Board of the "Policlinico G. Rodolico-Vittorio Emanuele" Hospital of Catania, Italy, all consecutive pregnant women who referred to Obstetrics and Gynecology department for spontaneous vaginal delivery, between 1 January 2011 and 31 December 2018, were identified and considered into the analysis. Among eligible patients, patients with primary PPH were selected and considered as "cases"; patients without PPH were considered as "controls" according to the inclusion/exclusion criteria and included with a ratio 1:4 (**table I**).

Table 1. Inclusion and exclusion criteria

Inclusion criteria
<ul style="list-style-type: none">• Delivery between 37° and 41°+6 week of gestational age• Age between 15 and 45 years• Spontaneous vaginal delivery and caesarean section
Exclusion criteria
<ul style="list-style-type: none">• Uterine myomas and other uterus structural defects• Previous surgery at the uterus and/or at the genitourinary tract• Diabetes mellitus

- Hyperaldosteronism or other electrolytes disorders
- Maternal kidney disease
- Coagulation disorders
- Multiple pregnancy
- Foetal macrosomia
- Preeclampsia or eclampsia
- Chorioamnionitis
- Delay and complications in second stage of labour
- Previous postpartum haemorrhage
- Genital tract lacerations
- Extension of episiotomy
- Placental retention
- Uterine inversion
- Prolongation of third stage of labour
- Retained placenta
- Placenta accreta

DATA EXTRACTION

Data were collected by direct consultation of the hospital medical records, ICD codes n. 666/01/02/03. All information regarding the characteristics of the patients were retrospectively collected and inserted in a dedicated electronic database. This included age, personal medical/clinical and obstetric history, serum electrolytes (K⁺ and Na⁺ at least), risk factors for PPH, data for each pregnancy (delivery modes, delivery, and postpartum complications like PPH and pregnancy outcomes).

VARIABLES AND OUTCOMES

Sodium and potassium serum levels before the delivery were considered primary independent variables, whereas the product of sodium and potassium (Na*K) was evaluated as secondary independent variable. Although, hypokalemia is defined as a serum potassium level less than 3.5 mEq/L and hyponatremia is defined as a serum sodium level less than 135 mEq/L, the outcome of our study was the difference in absolute average serum level of these serum electrolytes between cases and controls, with no regards to a conventional electrolytes cut-off. We considered

episiotomy and perineal laceration as potential confounders of primary PPH so we included them in the multivariate analysis. The estimation of blood loss was quantified using calibrated under-buttocks drape folds.

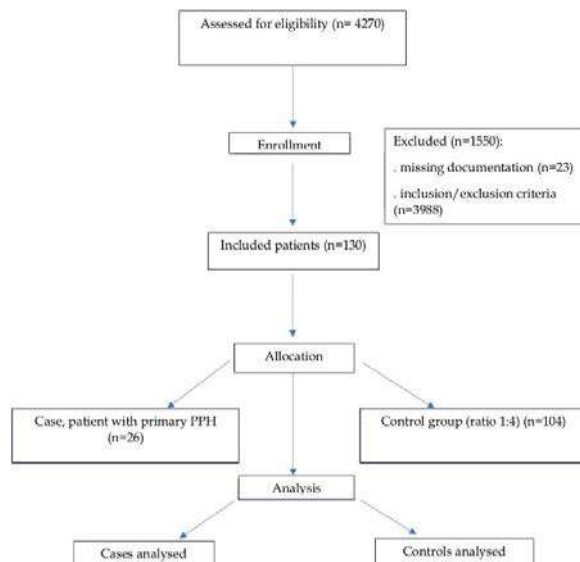
STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS for Windows (Statistical Package for the Social Science, version 21.0; SPSS Inc., Chicago, IL, USA). The level of significance was set at p <0.05. Absolute and relative frequency distribution as well as contingency tables were created. Continuous variables were expressed as median (Interquartile range). The differences between groups (cases and controls) were evaluated using non-parametric tests such as Chi-square and Mann-Whitney. Subsequently, a multivariate analysis was conducted, using a logistic regression model to analyze which risk factors were independently associated with PPH. To evaluate the best PPH predictive score, a ROC curve (Receiver Operating Characteristic) was used.

RESULTS

Among 4270 patients assessed for eligibility, only 130 patients were included in the analysis. Twenty-three patients were excluded due to data missing, whereas 3988 due to inclusion/exclusion criteria. Finally, we included in the study 26 cases with primary PPH and 104 controls (**figure 1**).

Figure 1 - Study flow chart.



Patients' characteristics were reported in **table II**. Vaginal delivery was slightly but statistically more present among the case group, whereas Cesarean section was slightly higher in the control group ($p=0.022$). There was no other statistical difference between the two groups.

Table II. Main characteristics of study population.

	Case (N=26)	Control (N=104)	p-value
Age	32.4 ± 2.5	31.9 ± 3.7	0.516*
Number of previous pregnancies			
None	14 (53.8)	45 (43.3)	0.338^
1 or more	12 (46.2)	59 (56.6)	
Number of previous pregnancies	3.2 ± 1.2	2.8 ± 1.4	0.183*
Mode of delivery method			
Vaginal delivery	22 (84.0)	62 (60)	0.022^
Cesarean section	4 (16.0)	42 (40)	
Apgar score at 5 minutes			
≥ 8	25 (96.2)	100 (97)	0.835^
≤ 7	1 (3.8)	4 (3)	
Blood transfusion (yes)	5 (19.2)	0 (0)	**

Data are expressed as mean ± standard deviation or as frequencies (percentages). ^Chi-square test

*Mann-Whitney test

**Due to the zero frequency, the p value is not provided.

Confounders, such as episiotomy and perineal laceration, show no statistical difference between the two groups (**table III**).

Table III. Distribution of the confounders by cases and controls.

Variable	Case (N= 26) n (%)	Control (N=104) n (%)	p-value*
Episiotomy			
Yes	8 (30)	35 (33.5)	0.735
No	18 (70)	69 (66.5)	
Perineal laceration			
Yes	1 (4)	15 (14.5)	0.148
No	25 (96)	89 (85.5)	

*Chisquare test.

The medians and the interquartile range of Na⁺, K⁺, and Na*⁺K, of both groups, were evaluated. Both the K⁺ level and the product Na*⁺K in pa-

tients with PPH were significantly lower compared to the control group $p<0.001$. Conversely, no differences were found between the two groups concerning the Na⁺ level (**table IV**).

Table IV. Medians and interquartile intervals of Na⁺, K⁺, and Na*⁺K.

Variable	Median (IQR*)			p-value**
	Total	Cases n= 26	Controls n=104	
Sodium (meq/L)	137 (135-139)	138 (134-139)	137 (135-139)	0.289
Potassium (meq/L)	4.1 (3.9-4.3)	3.8 (3.7-4.0)	4.1 (3.9-4.3)	0.000
Na* ⁺ K (meq/L)	554 (530-589)	524 (496-552)	557 (532-593)	0.000

*IQR: interquartile range ** U Mann-Whitney test.

We have found a positive association with potassium (OR=19, 95% CI 2.40-157) and Na*⁺K product levels (OR= 8.78, 95 % CI 1.96-39) (**table V**). Conversely, Na⁺ has not showed a statistically significant risk (data not furnished) to develop PPH. Due to the strong association between the low serum level of K⁺ and Na*⁺K product and the risk to develop PPH, the ROC curve was used in order to evaluate a possible diagnostic and predictive role of these electrolytes, especially for serum potassium level.

Table V. Odds ratio (OR) with 95% confidence interval (CI) of primary postpartum hemorrhage risk according to Na⁺, K⁺, and Na*⁺K product.

Variable	Case N= 26	Control N=252	OR (IC 95%)	OR adj1 (IC 95%)
Sodium (mEq/L)				
≥ 138	14 (53.8)	97 (38.5)	*	*
≤ 137	12 (46.2)	155 (61.5)	0.54 (0.24-1.20)	0.284 (0.093-0.867)
Potassium (mEq/L)				
≥ 4.2	2 (7.7)	108 (43.2)	*	*
≤ 4.1	24 (92.3)	142 (56.8)	9.13 (2.11-39.5)	19.40 (2.40-156.5)
Na* ⁺ K (mEq/L)				
≥ 555	4 (15.4)	133 (53.0)	*	*
≤ 554	22 (84.6)	118 (47.0)	6.2 (2.1-18.5)	8.78 (1.96-39.4)

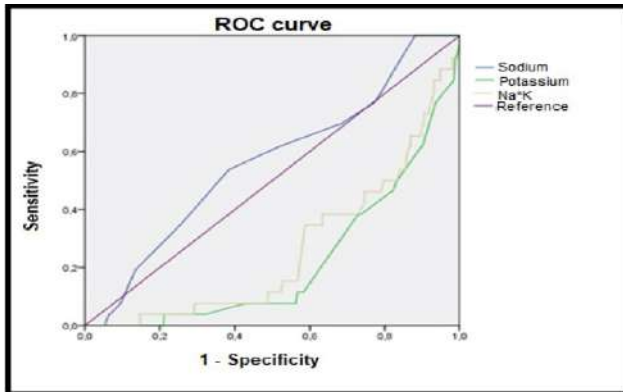
Data are expressed as frequencies (percentages).

*reference category: each odds ratio is adjusted for episiotomy and perineal laceration.

From the analysis of the ROC curve (**figure 2**), it can be seen that none of the electrolytes

investigated were able to discriminate between women who developed PPH or not, and therefore, they cannot be used as a diagnostic test.

Figure 2 - Receiver operating curve (ROC) of Na⁺, K⁺ and Na⁺ * K⁺ product serum levels as predictors of primary postpartum hemorrhage.



DISCUSSION

Our findings showed that reduced serum K⁺ levels could influence the risk of developing PPH. This point was not extensively analyzed before in the literature, although the role of potassium during pregnancy is widely studied (34–36). In fact, the altered potassium level in pregnancy was associated with an increased risk for atherosclerosis and is also a predictive factor for renal dysfunction, gestational diabetes mellitus and, hypertensive disorders (37–43). The myometrial contraction critically depends on the control of calcium entry through voltage-gated L- and T-type calcium channels (21,22). Calcium is not only an essential second messenger for contraction via calcium-calmodulin-dependent myosin light chain kinase, but also depolarizes the plasma membrane, with activation of other voltage-dependent ion channels (32,44). The control of uterine excitability is finely modulated during pregnancy. In particular, mid-gestation is characterized by the presence of hyperpolarized membrane potential close to the reversal potential for potassium E_k (45). At term, the myometrium becomes increasingly depolarized, to approximately 45 mV at parturition (23,45). This crucial mechanism remains unknown. Moreover, there has been a growing interest in the role of potassium channels such as Kir 7.1, an inwardly rectifying K⁺ channel, especially in the pathophysiology of postpartum hemorrhage (28,30). Kir 7.1 represents a crucial regulator of membrane potential in uterine myocytes during pregnancy: high expression of Kir 7.1 keeps the

resting membrane potential near to the reversal potential for potassium, thus increasing the depolarizing drive required to initiate an action potential, calcium entry, and subsequent contraction. At the term of pregnancy, this damping of excitability is lost by reduction but not a complete loss, of Kir 7.1. However, some authors found out the potential therapeutic benefit of inhibiting Kir 7.1 during oxytocin administration for postpartum hemorrhage (29,30,46,47). Serum potassium level is mainly regulated by renal potassium excretion, particularly by cells of the collecting duct. The factors that influence potassium excretion are high aldosterone level, high distal delivery of sodium to the collecting duct, and high negativity of the lumen in the collecting duct (48,49). Potassium metabolism during pregnancy is not fully understood. During a healthy pregnancy, the secretion of aldosterone is enhanced, and one of the causes is the increase of splanchnic and systemic vasodilatation (49). However, the high levels of progesterone act as mineral corticoid receptor blocker, and by that decrease, the full effect of aldosterone on the kidney and severe potassium wasting is avoided (31,49). During a healthy pregnancy, the glomerular filtration ratio (GFR) is modified (50–52). According to the literature, we evaluated the role of serum electrolytes during the third trimester of pregnancy, in order to identify a range of serum sodium levels that could act as a predictive risk factor for PPH. Our findings showed that reduced serum K⁺ levels, regardless of the range of values considered physiological outside of pregnancy, could influence the risk of developing postpartum hemorrhage. Na⁺ alone is not able to determine significant changes in uterine contractility and, therefore, at the risk of developing a PPH. However, our data are limited by the small number of patients selected in the case group due to the low frequency of the PPH in our setting. A multicentric retrospective study should be performed to understand the role of electrolytes in PPH better. Physiological changes during pregnancy influence normal biochemical values: for example, serum thyroid-stimulating hormone (TSH) value varies, especially during the first trimester, and no cut-off value is universally accepted. However, thyroid hormones are widely accepted as essential factors for the neurodevelopment of the children (53,54). The ORadj is adjusted odds ratio (AOR), further

confirming the role of serum electrolytes in the alteration of uterine contractility and in the risk of primary PPH. In our study, we also performed a ROC curve to evaluate the possibility of using the electrolytes as a possible diagnostic and predictive PPH test. However, the results showed that the electrolytes could not be used as a diagnostic test for PPH. The management of PPH remains a topic of great debate, and new diagnostic and therapeutic possibilities in recent years underline this aspect. However, the available evidence is still scarce and the stan-

dard values are lacking (12,14,55). This preliminary study focused on potassium as a crucial actor for myometrial contraction; however, it underlines the attention for serum values during the pregnancy to reduce the risk of PPH, also by considering oral supplementation.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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Hepatic mesenchymal hamartoma and placental mesenchymal dysplasia: an association ever less rare; a focus on current Knowledge

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ABSTRACT

Objectives. Hepatic mesenchymal hamartoma (HMH) is a lesion that originates from the mesenchymal tissue of the portal tract and that in most cases manifests itself in the first three years of life. The placental mesenchymal dysplasia (PMD) is a rare anomaly characterized by placentomegaly with the presence of hydropic chorionic villous that resemble those of hydatiform mole. In literature there is only a few number of cases characterized by this association that have led to formulate the hypothesis of a common pathogenetic pathway to the two pathologies. This report wants to offer tools for a correct prenatal diagnosis and management of similar cases.

Methods. We report a detailed description of the prenatal signs of association of a voluminous HMH and a PMD. Histological studies have been conducted on fetal tissues of hepatic and placental origin.

Results. The large size of the abdominal mass resulted in a severe impairment of respiratory function and the neonatal death. Histological study confirmed the prenatal diagnosis. The understanding of the etiopathogenetic mechanism of the association was negatively affected by the failure to carry out molecular genetic investigations on the affected tissues.

Conclusion. In light of current scientific findings, we recommend to always study the placenta very carefully in case of detection of fetal abdominal cystic masses, to plan molecular genetic investigations on affected tissues and to be very cautious considering the high incidence of neonatal complications and adverse outcomes.

SOMMARIO

Obiettivo. L'amartoma mesenchimale epatico (HMH) è una lesione che origina dal tessuto mesenchimale del tratto portale e che nella maggior parte dei casi si manifesta nei primi tre anni di vita. La displasia mesenchimale della placenta (PMD) è una rara anomalia caratterizzata da placentomegalia con la presenza di villi coriali idropici che assomigliano a quelli della mola idatiforme. In letteratura c'è solo un numero limitato di casi caratterizzati da questa associazione che ha portato a formulare l'ipotesi di una via patogenetica comune alle due patologie. Questo report vuole offrire strumenti per una corretta diagnosi prenatale e la gestione di casi simili.

Metodi. Riportiamo una descrizione dettagliata dei segni prenatali di associazione di un voluminoso HMH e un PMD. Sono stati condotti studi istologici su tessuti fetali di origine epatica e placentare.

Risultati. La grande dimensione della massa addominale ha provocato una grave compromissione della funzione respiratoria e la morte neonatale. Lo studio istologico ha confermato la diagnosi prenatale. Sulla comprensione del meccanismo eziopatogenetico dell'associazione nosologica ha influito negativamente il mancato allestimento di indagini genetiche molecolari sui tessuti coinvolti.

Conclusione. Alla luce delle attuali acquisizioni scientifiche raccomandiamo di studiare sempre con molta attenzione la placenta in caso di riscontro di masse cistiche addominali fetali; pianificare le indagini genetiche molecolari sui tessuti affetti e avere un comportamento molto prudente considerata l'alta incidenza di complicazioni e esiti avversi neonatali.

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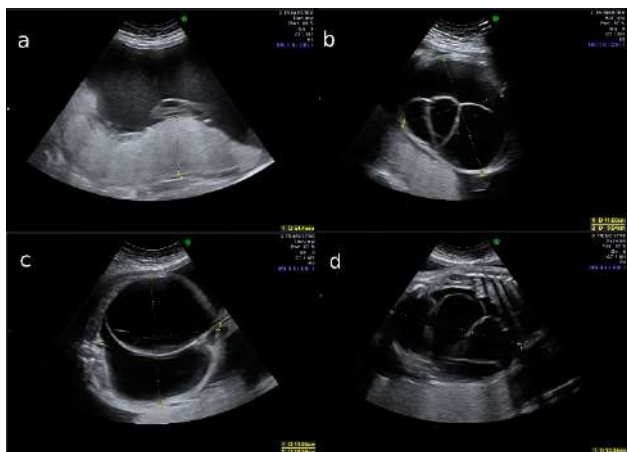
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Key words: congenital fetal tumors; hepatic mesenchymal hamartoma; hydatiform mole.

INTRODUCTION

A thirty years old woman, gravida 2 para 1, came to our observation at thirty weeks' gestation for the presence of a massive multicystic lesion in the fetal abdomen. The ultrasound examination showed a multiloculated cystic lesion of 128x115x99 mm which almost entirely occupied the fetal abdomen, extending from the inferior margin of the liver up to the pelvis. Ascites were not present. The color doppler examination did not show significant vascularization. It was associated with polydramnios and the placenta appeared thick and hyperechogenic (**figure 1a**). At the follow-up of the next week the mass was further increased in volume and the diagnosis of a HMH was suspected although the mainly exophytic development from the liver made difficult the differential diagnosis with other abdominal multiloculated lesions, such as mesenteric and omental cystic lymphangiomas (**figures 1b, c, d**).

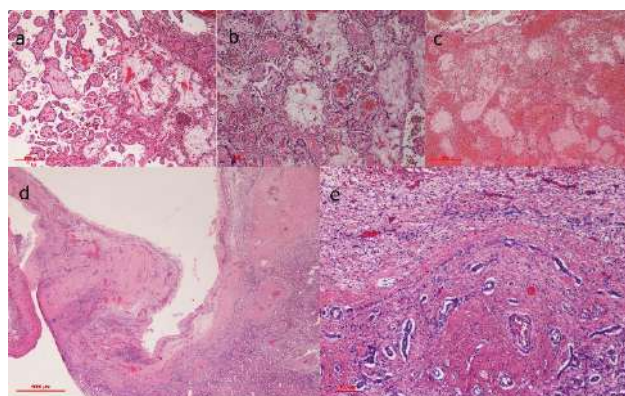
Figure 1. 2D image at 31 weeks' gestation showing a thickened and hyperechogenic placenta; (b-c) transverse and (d) parasagittal prenatal ultrasound image at 31 weeks' gestation showing a subphrenic multiloculated abdominal lesion.



At 32 weeks a mirror syndrome occurred for which it was decided to carry out the delivery by caesarean section. The large size of the mass resulted in severe impairment of respiratory function and despite intubation the newborn of female sex died three hours after birth. The autopsy was requested. Macroscopic examination of the placenta showed enlarged size (24x20x4,5 cm; 780 grams), eccentric insertion of the umbilical cord and an intraplacental hematoma (10 cm). Microscopically hydropic villous with dilated central cisterns (**figures 2a, b**), thick-walled

fibromuscular vessels, loose myxoid stroma and chorangioma like vasculature (**figures 2a, b**) were present. Moreover, intra and intervillous hemorrhage was appreciable (**figure 2c**). Autopsy revealed a wide (11x9 cm) multicystic lesion in the left lobe of the liver. Microscopically, the cystic walls were unlined, focally hemorrhagic and consisted of an admixture of mesenchyme and bile ducts (**figures 2d, e**).

Figure 2. Hydropic villi with dilated central cisterns and chorangioma-like vasculature. Hematoxylin-Eosin. Original magnification a: 2X, b:10X; (c) intra and intervillous hemorrhage. Hematoxylin-Eosin; original magnification 4X; (d-e) unlined cystic walls consisting of an admixture of mesenchyme and bile ducts. Original magnification a: 2X, b:10X.



DISCUSSION

HMH represents the second most common benign hepatic lesion of childhood (first two years of life) after hemangioma / hemangioendothelioma and clinically manifests itself with abdominal distension and / or as a mass of the upper abdomen. However, this condition is quite rare. There is a predominance in the male sex and about 75% of cases originate from the right lobe of the liver. Up to 20% develops from the lower surface of the liver therefore its hepatic origin could be difficult to determine as in the reported case. The HMH can reach considerable dimensions in childhood (1). It generally has a benign behavior and is susceptible to complete postnatal removal; however, rare cases of highly malignant tumor onset such as undifferentiated hepatic sarcoma on a previous HMH are reported. The origin of HMH is uncertain, although recent studies of molecular biology seem to indicate that it should be considered a benign neoplastic lesion ab initio (1). It has been diagnosed prenatally since the 19th week (2) but in most cases it manifests itself in the third

trimester. Ultrasound generally reveals a multicystic lesion with thick septa, more rarely with a mixed structure (solid and cystic) or completely solid. All lesions diagnosed during the prenatal period are hypovascular to the color doppler. This feature allows the differential diagnosis from the hepatic hemangiomas. Approximately 50% of cases have rapid growth in utero, the remaining tend to remain stable and in about 10-15% of cases may also undergo partial regression. The prognosis of the HMH diagnosed in the prenatal age is worse than those diagnosed in postnatal age. In the larger hamartomas the occurrence of fetal hydrops is frequent and is associated with a high perinatal mortality, as in the reported case. The pathogenetic mechanism of hydrops is unclear because, as we have already pointed out, hypervascular hamartomas have not been found in prenatal age. Kamata et al. (3) suggested that hydrops could depend on circulatory insufficiency due to rapid accumulation of fluid in cysts but is more likely to be related to compression of the inferior vena cava and / or umbilical vein. There is also an increase in the risk of preterm birth linked to the polydramnios which may require an amnioreduction (4). Intrauterine therapy may be indicated in selected cases as the appearance of hydrops at early gestational age. In these cases, the aspiration of the larger cysts can be performed. As the liquid tends to quickly regenerate it is often necessary to repeat the procedure in order to postpone the delivery. Alternatively, a cystic-amniotic shunt can be inserted which allows partial decompression of the lesion.

In our case the association between a HMH and a rare placental pathology represented by the PMD has been observed. This pathology was described for the first time by Moscoso et al in 1991 (5) and is characterized by placentomegaly and by the presence of cluster vesicles similar to those of the hydatiform mole. In rare cases the formation of the vesicles is minimal or absent. Differently from the hydatiform mole there is no trophoblastic proliferation. In the third trimester the chorionic plate reveals dilated and thick-walled vessels and various degrees of endoluminal thrombotic obliteration. Ultrasound in the second trimester shows a thickened placenta with multiple hypoechoic areas, similar to those of the hydatiform mole. As the pregnancy

proceeds, the cystic spaces are superficialized towards the chorionic plate and may not be evident on the ultrasound examination. In the described case the pregnant woman has come to our observation belatedly and we were not able to indicate how the placental structure was in the second trimester. The placenta in the third trimester appeared thickened and hyperechogenic but there were no cystic spaces in its context. Differently from the partial molar pregnancy, PMD is associated in most cases with a phenotypically normal fetus and pregnancy continues until the third trimester. PMD is associated with an increased risk of growth restriction, intrauterine death, preterm birth and some genetic abnormalities. In literature the incidence of intrauterine growth restriction ranges from 33% (6) to 50% (7), the intrauterine mortality from 13% to 40%. In about 50% of cases a preterm birth occurs (6) In about 20% of cases PMD is associated with the Beckwith-Wiedemann syndrome. More rarely chromosomal abnormalities, especially on the X chromosome (about 3%), were observed (8). To our Knowledge there are nine other reported cases characterized by this association that led to formulate the hypothesis of a common pathogenetic pathway to the two entities (2, 9, 10, 11, 12, 13, 14, 15). The most accredited theories are: (i) a congenital malformation of mesoderm; (ii) a dysregulation of genes implicated in the Beckwith-Wiedemann syndrome and (iii) an androgenic/biparental mosaicism (12, 16). According to the first main hypothesis PMD could originate from an inadequate blood supply which results in both aneurysmal dilatation of the chorionic vessels and a placental stem villous hyperplasia. As a result of thrombosis of the chorionic vessels, hepatic ischemic lesions would occur with a proliferative response of the bile ducts. Placental alterations arise during the second trimester and precede the development of the hepatic ones. On the basis of these observations, PMD would therefore represent a congenital anomaly of the mesoderm.

Recent literature also suggests the presence of multiples molecular mechanisms for C19MC gene activation on chromosome band 19q 13.4 in HMH and the existence of a placental imprinting pattern in mesenchymal cells (17). C19MC is maternally imprinted, so the placental expression is due to the paternal allele. C19MC is normally

expressed in the placental stroma and in case of androgenic/biparental mosaicism an association with the proliferation of stromal cells, similar to that of the HMH, has been noted (17).

The really incidence of androgenic/biparental mosaicism is unknown. Several mechanisms have been proposed to explain its origin in humans. Phenotypic manifestations can be very different due to the variable degrees of mosaicism and the combinations of uniparental disomy phenotypes.

A recent work emphasizes the importance of carrying out molecular genetic investigations, such as nucleotide polymorphism microarray and short-tandem repeat analysis, on the affected tissues to formulate an accurate genetic diagnosis (16).

In our case the anatomo-pathological and histological diagnosis were not supported by molecular genetic investigations targeted on the sample

tissues. This has perhaps subtracted information from the etiological understanding of the disease.

CONCLUSION

To date in literature there are only a few reports of association between the HMH and the PMD. Immunohistochemical and cytogenetic studies have led to formulate the hypothesis of a common causal association between the two pathologies. In light of the current acquisitions we can recommend to always study very carefully the placenta in case of finding of fetal abdominal cystic masses; not to stop at the conventional karyotype surveys but to plan molecular genetic investigations on affected tissues and to have a very heedful behavior considering the high incidence of complications and adverse outcomes.

CONFLICT OF INTEREST

The authors report no conflict of interests.

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Case report of prenatal diagnosis and surgical treatment of congenital ranula

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ABSTRACT

Congenital cystic pathologies of the mouth are very rare. The term "ranula" describes a swelling in the floor of the mouth, caused by a mucous extravasation cyst (MEC) or, less commonly, a mucous retention cyst (MRC); it derives from the main sublingual or sub-mandibular salivary glands. We describe a case of congenital mucous cyst of the mouth's floor, diagnosed in-utero by ultrasound in a fetus at 27 weeks' gestation. Ultrasound scan follow-up revealed no changes in the size or position of the cyst. The fetal growth was normal, as the amniotic fluid volume. Surgical treatment was performed five days after delivery. There were no complications and no recurrences occurred to now. The diagnosis was confirmed by postnatal histology.

SOMMARIO

Le patologie cistiche congenite della bocca sono molto rare. Il termine "ranula" descrive un gonfiore nel pavimento della bocca, causato da una cisti da stravasamento di muco (MEC) o, meno comunemente, da una cisti da ritenzione di muco (MRC); deriva dalle principali ghiandole salivari sub-linguali o sottomandibolari. Descriviamo un caso di cisti mucosa congenita del pavimento della bocca, diagnosticata in utero tramite ecografia in un feto alla 27^a settimana di amenorrea. Il follow-up ecografico non ha rivelato cambiamenti nelle dimensioni o nella posizione della cisti. La crescita fetale era normale, come il volume del liquido amniotico. Il trattamento chirurgico è stato eseguito cinque giorni dopo il parto. Non ci sono state complicazioni né recidive avvenute fino ad ora. La diagnosi è stata confermata dall'esame istologico postnatale.

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Key words: *Ranula; sublingual cyst; prenatal diagnosis; ultrasound scan*

INTRODUCTION

Congenital cystic pathologies of the mouth are very rare. The term "ranula" describes a swelling in the floor of the mouth, caused by a mucous extravasation cyst (MEC) or, less commonly, a mucous retention cyst (MRC); it derives from the main sublingual or submandibular salivary glands (1). MECs develop by disruption of minor salivary gland ducts and subsequent extravasation of mucous secretions into the contiguous connective tissue, whereas MRCs develop by proximal expansion of a blocked duct. MECs mostly occur in children and young adults, rarely in newborns. The main difference between the two abnormalities is that, differently to MECs, MRCs are lined by epithelium. The incidence of congenital ranula is about 0.74% (2).

Clinically the fluid-filled pseudocyst elevates the tongue. These pseudocysts are normally sited in the sublingual space between the mylohyoid muscle and the lingual mucosa. Occasionally, the edema produced by its growth extends into the submental or submandibular space through the mylohyoid muscle. The differential diagnosis of ranula are lymphatic malformations and fetal cervical tumors, such as teratomas, thymomas and thyroid tumors (3). An appropriate diagnosis is extremely important, as treatment and prognosis of both conditions are quite different (4). International scientific literature reports only few cases of prenatal diagnosis of ranula. We describe a case of congenital mucous cyst of the mouth's floor, diagnosed in utero by ultrasound in a fetus at 27 weeks' gestation, and confirmed by postnatal histology. This case report describes also the postnatal surgical treatment of this abnormality. The main novelty of this case report is represented by the fact that an accurate ultrasound prenatal diagnosis based on the size of the ranula allows us to know in the antenatal period (and confirm after birth) that was not necessary to perform an ex utero intrapartum treatment (EXIT) procedure, and we could wait five days for surgical operation.

CASE REPORT

A 29-year-old Caucasian woman, gravida 1, para

0, with a history of two previous surgical treatments for truncus arteriosus, came to our observation to perform a routine second-trimester fetal ultrasound scan at 27 weeks' of gestation. The scan showed a single viable intrauterine fetus with a hypoechoic mass in the floor of the mouth. The cyst was sited below the tongue and ahead to the inferior part of the maxillary bone. It measured 28 mm (length) and 21 mm (width). The margins were clear and no echoes could be observed inside the mass (**Figure 1-2**).



Figure 1. Ultrasound diagnosis of ranula



Figure 2. Ultrasound diagnosis of ranula

The tongue was displaced upwards, but all other findings were within normal limits. The tongue moved normally and the fetus appeared to swallow normally. Color Doppler imaging showed no neovascularization, either inside or outside the cyst.

She had run the first trimester screening for aneuploidies with negative results and even after the diagnosis of sublingual cyst, she decided to not perform amniocentesis.

Two follow-up ultrasound scans were performed at 31 weeks and at 36 weeks of gestation, and no changes were observed. The fetus showed normal development, the cyst did not change in size or consistency and there was no evidence of polyhydramnios. Cesarean section was performed at 40 weeks' gestation for premature rupture of membranes and fetal breech presentation. A normal 2900 g female baby was born with an Apgar score of 9 at 1 min and 10 at 5 min. The infant had a 40 mm sublingual cystic mass (**Figure 3**) which displaced the tongue upwards.

Her gag reflex was normal. Infra buccal and cervical prolongations were not found. Five days after delivery, a cyst's marsupialization was performed, and a new excretory duct of the gland was surgically created.



Figure 3. Surgical treatment of Ranula

The cyst was surgically drained under general anesthesia (**Figure 4-5**). The fluid obtained was mucus-like and the final pathology was identified as a mucosal retention cyst. After 36 hours, the baby quickly resumed a normal breastfeeding and his aesthetic appearance was satisfactory. The patient was discharged 6 days after the surgical operation. At the discharge, a soft tissue edema (false Ranula) was still present, but it was solved spontaneously within 7-10 days. After one month, the clinical follow up showed no evidence of recurrence.

The study protocol was approved by the Ethics Committee of the ARNAS Garibaldi Hospital and conformed to the ethical guidelines of the Helsinki Declaration. The woman signed an informed consent before entering the study, and her anonymity was preserved.



Figure 4. Surgical treatment of Ranula

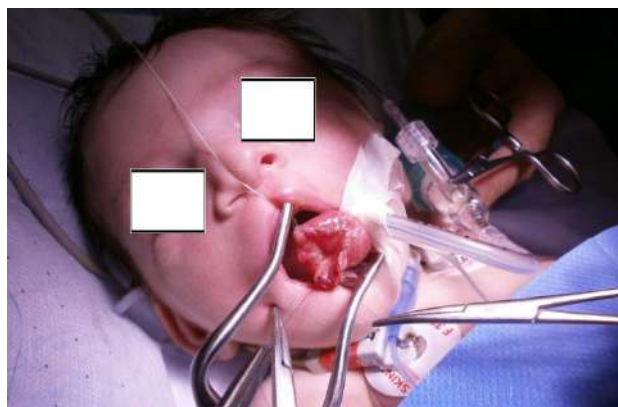


Figure 5. Surgical treatment of Ranula

DISCUSSION

A classification of the ranulas could be done according to their localization. Simple ranulas are sited in the mouth's floor, cervical ranulas in the paracervical area, and plunging ranulas near the superior airway. Plunging ranulas could extend into the floor of the mouth, and the histopathology is the only way to differentiate them by other types. Ultrasonographically they are indistinguishable.

The best surgical treatment is a two steps operation based on marsupialization, and subsequent posterior resection (1). The differential diagnosis of oral cystic abnormalities involves other exophytic congenital lesions. This group includes epignathus, gingival cyst of the newborn, palatal cyst of the newborn, congenital epulis, vascular hamartomas and lymphangiomas. Another pathology that has to be ruled out is the oropharyngeal teratoma, which is an uncommon congenital tumor, associated to significant morbidity and mortality.

These abnormalities distort the orofacial anatomy and often cause respiratory distress at birth. An optimal management of these conditions requires prenatal diagnosis by ultrasound. The teratoma appears as a solid-cystic tumor with mixed areas of hypo- and hyperechogenicity and it is usually found in association with polyhydramnios. Posterior magnetic resonance imaging has to be subsequently performed to confirm the diagnosis.

Finally, the differential diagnosis of cystic pathologies of the tongue includes thyroglossal duct cyst, with an unusual localization in the mouth's floor, congenital abnormalities of the submandibular duct, heterotopic gastric cyst and enterocystoma (3).

All of these conditions are rare, with a similar hypoechogenic ultrasound pattern, in which magnetic resonance imaging could be helpful to differentiate them (4-5).

Large size ranulas can cause airway obstruction with hypoxia at birth and hind the movements of swallowing causing polyhydramnios. In the massive ranulas, when there is the risk of hypoxia caused by an airway obstruction, the so-called ex utero intrapartum treatment (EXIT) procedure is required (6-7).

This procedure, by partial drainage of the cyst, needs to maintain airway before that fetomaternal circulation is interrupted.

The elective treatment of ranulas is the surgical treatment, and it may have different approaches: aspiration of the cyst, gland marsupialization, excision of ranula, and excision of ranula and ipsilateral salivary gland, crioechexesi.

The airway management of the newborns is

extremely difficult to maintain, particularly in case of large ranulas, so it has to be considered when these surgical interventions have to be planned (8). Prenatal diagnosis of a cystic lesion of the oral cavity, if carried out, requires a careful differential diagnosis to determine appropriate management of delivery and perinatal care. The main decision to consider is related to the size of the ranula: in case of bigger ranulas it has to be decided if perform the ex utero intrapartum treatment (EXIT) procedure or wait and make it in a second time (9-10). In our case report the fetal airway were not compromised at birth, as we preannounced by prenatal ultrasound diagnosis, so it was decided to perform the operation after 5 days.

CONCLUSION

Ranula is an unusual finding. Definitive diagnosis is obtained only after histological examination. When a prenatal cyst in the mouth is diagnosed, an adequate follow-up is necessary to assess the size and potential growth of the cyst, as well as all possible associated complications. According to this, the time of surgery has to be decided accurately, as well as the need for immediate action to ensure airway of the newborn after delivery. An accurate prenatal ultrasound diagnosis, based on the size of the cyst (as described in our case report) could be extremely important to orient the time of surgery.

CONFLICT OF INTEREST

The authors report no conflict of interests.

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