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REPRODUCTION AND CHILDBEARING HEALTH: REAL-WORLD EVIDENCE FROM ADMINISTRATIVE DATABASES

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ABSTRACT

Introduction

Over the last three decades, especially in high-income countries, reproduction has undergone significant changes, influenced by a combination of social, economic, technological, and cultural factors. The delay in the age at which individuals and couples decide to have children is one of the most important changes. In this context, addressing the new clinical and sociological challenges requires comprehensive health and public policy action.

Objectives

The main objective of this work was to monitor reproductive and childbearing health. The various projects identified conditions and factors affecting the health of children and mothers from an epidemiological, clinical, social and pharmaco-epidemiological point of view. In particular, the impact of pregnancy-associated cancers on pregnancy and birth outcomes, the consequences of advances in assisted reproductive technologies, and patterns of drug prescription during pregnancy and postpartum were the main research questions.

Materials & Methods

Sources. Regional administrative databases on health care utilisation are large repositories of data on health care systems that are routinely collected by health care providers and other institutions. For the purposes of the projects carried out, data were obtained from the following databases: i) an archive of beneficiaries of the regional health service, ii) the hospital discharge register, iii) the outpatient drug prescribing register, iv) the outpatient specialistic visits register, and v) the certificates of delivery assistance (CeDAP) database. Particularly, CedAP is the richest source of health, epidemiological, and socio-demographic information on births at national level and is an essential tool for for planning and supporting public health policies related to childbirth at national and regional levels.
Design. Historical population-based cohort studies. The birth cohorts were identified from the hospital discharge register and CedAP. The different databases were linked to each other with record linkage technique, an algorithmic procedure whose purpose is to identify and merge records from one or more data sources that correspond to the same individual.
Statistical analysis. Standard descriptive statistics, including frequencies and percentages for categorical data and means and medians for quantitative data, were calculated to assess the distribution of maternal sociodemographic and clinical characteristics. The Chi-square test and absolute standardised differences were used when appropriate for testing differences in categorical variables. Independent samples t-test was used to

compare numerical variables. Logistic or log-binomial regressions were fitted to estimate the odds ratio or prevalence ratio and 95% confidence interval (95% CI) of specific outcomes associated with specific exposure approached in each study. Models were adjusted for potential confounders. Ad hoc analyses were performed for single studies.

Results

Pregnancy-associated cancer. A first study aimed to assess the association between a diagnosis of pregnancy-associated cancer and adverse perinatal outcomes. It found that women with oncological condition tended to have a higher incidence of iatrogenic preterm birth. The need for timely and appropriate medical interventions to manage both the cancer and the pregnancy can sometimes lead to induced preterm birth even in the absence of a specific oncological indication, compromising the wellbeing of the newborn.

The second study, which investigated the impact of a cancer diagnosis on pregnancy outcome, found a downward trend in the number of abortions in favour of live births. Advances in research and clinical practice, together with increased awareness of the potential of treating oncological conditions in pregnant women, have contributed to this trend.

Assisted reproductive technologies. The relationship between assisted reproductive technologies and twinning rates and adverse perinatal outcomes, such as preterm birth and birth defects, has been investigated in a number of studies. The results showed a notable decrease in multiple pregnancies, which was attributed to the increasingly common practice of single embryo transfer. This approach is consistent with a focus on reducing the risks and challenges often associated with multiple pregnancies, including preterm birth.

Other analyses with a sociological perspective have shown that little attention has been paid to the contribution of assisted reproduction to desired family size, with the main objective remaining to reduce childlessness.

Prescribing patterns during pregnancy and postpartum. More and more women may need to be treated with medication during pregnancy, monitoring of medication prescription patterns during pregnancy could be a tool for assessing compliance with recommended supplement and medication use in clinical practice. An overview of drugs prescriptions was provided.

The study focused on initiation of antidepressant use during postpartum found that mothers who experienced a preterm birth had an excess risk of antidepressant use, suggesting an association between preterm birth and maternal mental illness.

Conclusion

It is essential that attention and resources are focused on continuous and close monitoring of reproductive and childbearing health to promote and ensure healthy pregnancies, safe childbirth, and the well-being of mothers and couples.

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CHAPTER 1 - INTRODUCTION

1.1 Rationale

Reproductive habits and the process of childbirth are in a constant state of flux, and it is of the paramount importance to monitor these aspects in order to be able to respond to the various concerns of the clinical and sociological debate, as well as to improve health care services. In this thesis, administrative databases on health care utilisation are used to monitor selected conditions that affect mothers and newborns. Analysis of this data will be used for exploration and improvement of reproductive health.

First of all, there is the vastness of information that health care utilization databases can be a source of. Administrative data provide a comprehensive and easily accessible source of information covering a wide range of birth-related variables, such as maternal demographics, birth outcomes, and neonatal health indicators. This wealth of data and the ability to explore the relationships between the available variables, enables health authorities and researchers to gain a holistic understanding of the birth process and its outcomes at both individual and population levels.

The systematic collection of administrative data allows for the timely detection of trends, patterns, and potential issues in childbirth practices and outcomes. By analyzing this data, healthcare providers and policymakers can identify areas where improvements are needed, implement targeted interventions, and track the effectiveness of these interventions over time. This proactive approach can significantly enhance the quality of maternal and neonatal care.

Overall, the monitoring of births using administrative data is essential to promote evidence-based decision-making, improve the quality of care, reduce health inequalities and improve the overall health and well-being of mothers and newborns. It plays a pivotal role in improving maternal and neonatal health outcomes and promoting a health system that is responsive to the needs of diverse populations.

In addition, administrative data make it possible to reconstruct the characteristics of mothers according to different demographic factors, such as race, ethnicity, socioeconomic status and geographical location, and to examine how these disparities may affect inequalities in access to maternity care and health outcomes. Taking into account the available clinical aspects, it is also possible to reconstruct the medical history and classify mothers into risk categories in order to identify high-risk categories requiring further care.

The concept of motherhood is undergoing significant changes from both a social and clinical perspective, mainly due to the increasing trend of delayed motherhood. This shift in maternal age has brought with it a number of challenges, including increased difficulty in naturally achieving pregnancy and an increasing number of pregnant women with co-morbidities. As a result, there is an increasing need to strike a delicate balance between maternal health and the well-being of the developing foetus and the pregnancy itself. This issue is particularly acute in cases where women face conditions such as oncological disease or chronic medication use. The changing landscape of

motherhood underscores the importance of comprehensive healthcare that addresses not only the unique needs of expectant mothers, but also the complexities that arise from the intersection of maternal health and the evolving clinical landscape.

The main topics explored in this thesis are: pregnancy-associated cancers (PAC), assisted reproductive technologies (ART), and aspects of pharmacoepidemiology. First, pregnancy-associated cancers is a concern of utmost human and social importance, as well as a complicated clinical scenario requiring an integrated and multidisciplinary approach. Given the difficulty of addressing both maternal and fetal health, a cancer diagnosis made during pregnancy may have an impact on a woman's course of treatment and chance of survival. Second, given the significance of infertility and its effect on people's quality of life and general wellbeing, it is critical to address the clinical and sociological issues surrounding it. ART represent an efficacious and increasingly adopted solution against this condition. Finally, understanding the rational use of drugs in pregnancy and exploring patterns of use is crucial and monitoring drug prescription patterns during pregnancy could represent a tool of utmost importance to evaluate the adherence of clinical practice to recommended consumption of supplements and medications.

1.2 Objectives

Reproductive health monitoring is the main objective of the investigations presented in this thesis, which identifies conditions and factors affecting the health of children and mothers from an epidemiological, clinical, social and pharmacoepidemiological point of view.
To achieve this goal, temporal trends in crucial conditions were considered and the association between selected maternal exposures and various obstetric and neonatal outcomes was assessed.

Specific objectives are briefly described below and detailed in the relevant section of each study.

I Study - To assess the association between the diagnosis of pregnancy-associated cancer and adverse perinatal outcomes;
II Study - To evaluate if the risk of abortion increases in women diagnosed with cancer;
III Study - To describe monozygotic twinning rate after assisted reproductive technologies;
IV Study - To assess the association between assisted reproductive technologies and preterm birth;
V Study - To investigate the prevalence, potential risk factors, and consequences of birth weight discordance;
VI Study - To evaluate impact of assisted reproductive technology on the second birth;
VII Study - To evaluate long-term impact of COVID-19 pandemic on births after assisted reproductive technologies;
VIII Study - To provide an overview of drug prescription patterns during pregnancy;
IX Study - To explore the relationship between preterm birth and the risk of the initiation of antidepressants use in the year after birth.

CHAPTER 2 - LITERATURE REVIEW

2.1 Literature review of pregnancy-associated cancers

PAC are neoplasms diagnosed during pregnancy or within one year of delivery or abortion. Tumors diagnosed in the first year after the end of pregnancy are assumed to have originated in the preceding months during pregnancy. The symptoms associated with the malignancy may be due to the physiological changes of pregnancy or may not have been detected early because they are mild, and the diagnosis by instrumental examinations may have been delayed because during the months of pregnancy and lactation, exposure to procedures is often avoided in the absence of a strong indication.
The diagnosis of a malignant neoplasm during pregnancy or in the first year postpartum is an event of great human and social significance, involving complex clinical management and requiring an integrated strategy by a multidisciplinary team. It is therefore important to define the magnitude of this event through the incidence rate and to assess its impact.
As the understanding of PAC continues to evolve, so does the need for tailored approaches to risk assessment, early detection and therapeutic interventions that can optimise outcomes for both the mother and the developing fetus.

2.1.1 Epidemiological framework

Despite an abundance of evidence, findings on the prevalence of PAC remain poorly comparable. This is due to differences in study design and methodology, varying inclusion criteria, inconsistent follow-up periods, and diverse reference populations. PAC affects approximately 1 in 1000 pregnancies [1].
Table 1 lists several epidemiological studies carried out in different geographical areas at world, European and Italian level. Specifically, the population of interest, the sample size and incident cases, the postpartum observation period, and the incidence of malignancies are described. The incidence measures ranging from 0.65 per 1000 in Finland to 1.73 per 1000 in Korea.
The frequency of PAC observed in the population Lombardy is largely in line with data reported by studies carried out in the rest of the world: a recent research examining pregnancies between 2002 and 2011 reports an incidence of 1.23 per 1000 pregnancies [2]. Another study conducted also in Italy specifically in Apulia, estimates an incidence of 1.27 per 1000 pregnant women [3].
Pregnant women are more likely to experience cancers that are more common in women of reproductive age, with an incidence generally similar to that of women of the same age who are not pregnant [4].

Table 1. Result from selected studies about pregnancy-associated cancer (PAC).

		In the world		
Author, study period	Smith [5], 1992-1997	Lee [4], 1994-2008	Shim [6], 1995-2013	Cottreau [7], 2001-2013
Geographical area	California	Australia	Korea	Stati Uniti
Population	Births	Births	Pregnancies	Pregnancies
Incidence (per 1000)	0.71	1.37	1.73	1.09
		In Europe		
Author, study period	Nieminen [8], 1950-1969	Lundberg [9], 1973-2017	Eibye [10], 1977-2006	
Geographical area	Finland	Sweden	Denmark	
Population	Pregnancies	Births	Pregnancies	
Incidence (per 1000)	0.65	1.02	0.90	
		In Italy		
Author, study period	Parazzini [2], 2001-2012	Murgia [3], 2003-2015	Pierannunzio [11], 2003-2015	
Geographical area	Lombardy	Apulia	Several regions in North, Centre, and South	
Population	Pregnancies	Pregnancies	Pregnancies	
Incidence (per 1000)	1.23	1.27	1.24	

2.1.2 The challenge of diagnosis

Early diagnosis of cancer is essential for successful treatment, regardless of pregnancy status. Unfortunately, the diagnosis of cancer in pregnancy is often delayed. Diagnosis in pregnancy is made more difficult by the fact that many of the symptoms of malignancy are similar to the symptoms of pregnancy, including nausea, breast changes, abdominal pain, anaemia and fatigue. Breast changes and the gravid uterus can make the physical examination of a pregnant woman difficult.

Altered hormone levels may cause changes in the breast that can hide small new formations. Women, but also clinicians, may misinterpret these changes, confusing physiological changes with early signs of neoplasia and delaying diagnosis by a few months [12,13]. Similarly, the hyperproliferative state of the breast can lead to a relatively high rate of false positive cancer diagnoses [13,14]. Therefore, although biopsy of a suspected breast lesion can be problematic due to the hypervascularisation and oedema typical of the gravid state, it should be considered a more sensitive procedure for accurate diagnosis [13].

Hyperpigmentation characteristic of pregnancy may also have an impact on the interpretation and manifestation of possible malignant skin lesions or melanoma [12,13].

On the contrary, pregnancy favours early diagnosis of cervical cancer: inspection, cytological examination, and bimanual palpation are routine care that the pregnant woman undergoes. The definite and specific diagnosis of this type of neoplasm is conducted by material sampling and biopsy with anaesthesia that can be performed safely during gestation [13]. In particular, it is shown that in pregnancy the probability of diagnosis of cervical cancer in the early stage, when the neoplasm is operable, is twice as high as in the general population. However, even in this case, the interpretation of cytological specimens obtained can be difficult: the physiological changes associated with pregnancy may lead to false-positive results and therefore the cytopathologist must be well informed; for example, a Papaniculaou smear obtained from a pregnant woman may show squamous metaplasia or trophoblastic cells that could be mistakenly considered dysplasia.

A large number of imaging techniques can be used as diagnostic methods in pregnancy. These techniques include ultrasound, ionising radiation, magnetic resonance imaging, and nuclear medicine. Ultrasound and magnetic resonance imaging can be used safely in pregnancy. A specific point must be made for x-rays and nuclear medicine: the diagnosis and staging of cancer using instrumental techniques must in fact limit the exposure of the patient and the foetus to ionising radiation. Specifically, during the first trimester of pregnancy, only strictly necessary radiological examinations would be justified. Other diagnostic procedures such as incisional or excisional biopsies, endoscopies and bone marrow punctures can be safely performed. It is essential to remember that if a diagnostic procedure is medically indicated, the mother's risk of not undergoing it is greater than the risk of harm to the foetus [13,15].

2.1.2.1 Diagnostic methods

The American College of Obstetricians and Gynecologists (ACOG) has drawn up guidelines for the use of x-rays, ultrasound, magnetic resonance imaging and radioisotopes during pregnancy and lactation (**Table 2**) [15]. Imaging techniques are important diagnostic procedures for acute and chronic conditions now rooted in traditional medicine, yet there is still confusion about their use and safety in pregnancy and lactation, so they are often avoided or accompanied by unnecessary interruption of breastfeeding.

Table 2. American College of Obstetricians and Gynecologists (ACOG) guidelines for the use of x-rays, ultrasound, magnetic resonance imaging and radioisotopes during pregnancy and lactation.

"Ultrasonography and magnetic resonance imaging (MRI) are not associated with risk and are the imaging techniques of choice for the pregnant patient, but they should be used prudently and only when use is expected to answer a relevant clinical question or otherwise provide medical benefit to the patient."
"With few exceptions, radiation exposure through radiography, computed tomography (CT) scan, or nuclear medicine imaging techniques is at a dose much lower than the exposure associated with fetal harm. If these techniques are necessary in addition to ultrasonography or MRI or are more readily available for the diagnosis in question, they should not be withheld from a pregnant patient."
"The use of gadolinium contrast with MRI should be limited; it may be used as a contrast agent in a pregnant woman only if it significantly improves diagnostic performance and is expected to improve fetal or maternal outcome."
"Breastfeeding should not be interrupted after gadolinium administration."

Source: [15]

2.1.3 Clinical management: a multidisciplinary approach

When addressing the clinical management of a pregnant patient with cancer, a comprehensive assessment is essential. It involves analyzing the intricate interplay between the progression of the tumor and the decision regarding the continuation of the pregnancy. Factors such as the stage and type of cancer, its potential impact on both maternal and fetal health, and the available treatment options that can be administered safely during pregnancy need to be thoroughly evaluated. Equally important is understanding how the ongoing pregnancy might influence the approach to tumor treatment, considering the safety of the fetus and the potential adjustments required in the treatment plan to ensure the best possible outcomes for both the mother and the baby.

The clinical management of a oncological pregnant patient is certainly a complex scenario due to the co-presence of mother and foetus: an accurate diagnosis and timely treatment can save the mother's life, but have irreparable consequences for the foetus. Oncological illness in pregnancy also carries with it a strong psychological impact involving the woman, the couple and the family. The management of these situations requires a shared strategy by a multidisciplinary team of gynaecologists, oncologists, surgeons, radiologists, neonatologists, psychologists, and other specialists [16]. As for non-pregnant women the approaches to cancer are: radiotherapy, chemotherapy and surgery [13]. The choice of treatment and its timing is a challenge for each member of the medical and support team and cannot be separated from the maternal and couple's wishes [16].

2.1.3.1 Surgical treatment

The planning of surgery during pregnancy is based on an assessment of the potential risks to mother and foetus. Maternal surgery with a fetus in utero may result in premature labour and altered utero-placental perfusion with consequent risk of hypoxia, brain injury, and fetal endouterine death. Delayed maternal surgery may, however, lead to progression of maternal disease and risk of fetal metastases [17].

Despite the risks mentioned, the evidence is rather reassuring. A systematic review considered over 12,000 women who underwent non-obstetrical surgery during pregnancy between 1966 and 2002 and found no increased rate of miscarriage or adverse birth events compared to the general population [18].

As regard anaesthesia, the Federal Drug Administration suggests minimising the time under general anaesthesia, minimising the dose and concentration of the agent and avoiding inhalation anaesthetics, propofol, and midazolam [19]. However, no data is reported in the literature with regard to the effects on cognitive development from in utero exposure to maternal general anaesthesia for non-obstetrical interventions [17].

2.1.3.2 Radiotherapy during pregnancy and lactation

Radiation doses used in cancer therapy are usually in the range of 4000-7000 cGy, which is more than 1000 times the level of diagnostic radiology. However, fetal exposure depends on several factors, including the target dose, the size of the radiation fields and the distance from the edges of the fields to the fetus. Generally, a distance of more than 30 cm from the edges of the field will produce a fetal exposure of only 4-20 cGy, so many areas such as the head, neck and extremities can be treated with radiation without fetal exposure [20].

Based on this evidence, recent studies state that radiotherapy, when strongly recommended, can be used safely in pregnancy as long as the uterus is out of the field and shielded from radiation. In particular, shielding can result in 58-71% reduction in foetal exposure dose. In addition, methods such as stereotactic ablative and intensity-modulated radiotherapy can ensure very low exposure.

As regard postpartum period, radiation therapy while breastfeeding presents unique challenges and considerations for both mother and baby. Radiation can pass into breast milk, potentially exposing the infant to radiation. However, the amount of exposure and its effect on the baby's health depends on several factors, including the type and location of the radiation treatment, the dose, and the timing of the treatment in relation to breastfeeding. In some cases, it may be advisable to temporarily stop breastfeeding or to express and store breastmilk before radiotherapy to ensure the baby's safety. Close consultation and cooperation between the medical team, including oncologists and paediatricians, is essential to make informed decisions that prioritise both the mother's cancer treatment and the well-being of the breastfeeding infant.

2.1.3.3 Chemoterapy during pregnancy and lactation

The administration of chemotherapy during pregnancy has been gradually increasing over the past two decades [21]. Chemotherapeutic agents block or attack cells during different phases of the cell cycle and are rarely used alone, rather they are combined to increase their antineoplastic efficacy [22]. This type of treatment is strongly discouraged in the first trimester of pregnancy, where the fetal risk of malformations is 10-20% with single drug treatment and rises to 25% with combination therapies. In the second and third trimesters the teratogenic risk falls, but the risk of premature birth, perinatal death and intrauterine growth restriction increases [17,22-25]. Few studies have looked at possible long-term neurological effects, but have shown no significant difference in cognitive abilities or school performance between children who had been exposed to chemotherapy in utero and their controls [23].

The impact of treatment on pregnancy and the foetus depends on several factors, such as the duration and timing of exposure, the dose that reaches the embryo or foetus, and how it interferes with cell metabolism [22,24]. Evidence reports that most chemotherapy drugs have low molecular weight, so they can potentially cross the

placenta and reach the foetal circulation [26], however not all drugs are equally dangerous [27,28].
In general, antimetabolites, small molecules used in the treatment of leukaemia, lymphoma and breast cancer, are considered to be the chemotherapeutic agents with the greatest teratogenic potential and their use is therefore not recommended during pregnancy [13]. On the other hand, alkylating agents are considered safe in the second and third trimesters; some teratogenic effect has also been reported for this family of drugs in the first trimester. These are drugs used to treat breast, ovarian and non-Hodgkin's lymphomas. The use of anthracyclines, although associated with cardiotoxicity, is not discouraged, but not all drugs in this group have been tested, so great caution is required. Vinca alkaloids are very similar to plasma proteins and appear to be less teratogenic than other chemotherapeutic agents. The use of taxanes has not been studied in sufficient numbers to make a firm judgement on their safety, and they are therefore not recommended during pregnancy. As chemotherapy is a pharmacological therapy, it should be emphasised that the physiological changes induced by pregnancy may affect the pharmacokinetics and pharmacodynamics, i.e. the absorption, distribution, metabolism, excretion and mechanism of action of the drug. These changes include an approximately 50% increase in plasma volume, renal clearance and hepatic metabolism. All this can lead to a reduction in the active concentration of the drug compared with the same dose given to a non-pregnant woman of the same weight. The above suggests that a higher dose of chemotherapy may be needed in pregnancy, but this is still unclear [22].
Also chemotherapy during breastfeeding presents a complex dilemma for both the mother and infant. Chemotherapeutic agents are known to pass into breast milk, potentially posing risks to the baby's health. However, most drugs used to treat cancer have almost no published information on their use in breastfeeding mothers or even measured concentrations in breast milk that can be used to measured in breast milk to assess their safety. Most breastfeeders are advised to stop breastfeeding during cancer treatment, as there are fears that the baby may suffer serious side effects. But suddenly stopping breastfeeding can cause psychological trauma for both mother and baby, and may not be necessary. A review reported a number of evidences about safety of metoclopramide, 5-fluoruracil, etoposide, and growth factors; on the contrary, for cisplatin, mitoxantrone, doxorubicin, and high-dose methotrexate, a high plasma-milk passage was observed [29].

2.1.3.4 Management from obstetricians' and gynecologists' view point

Pregnancy in a woman diagnosed with malignancy is considered a high-risk obstetric pregnancy, so it is desirable that it be monitored at a highly specialised centre with an efficient multidisciplinary team [16,30,31].
Regular and thorough ultrasound examination is considered important, and attention is focused on early detection of malformations, assessment of fetal growth, placental

flow, and amniotic fluid regularity, all indicators of fetal well-being and physiological course of gestation [16,30,31].

If a treatment programme against the neoplasm is planned, certain precautionary measures must be taken. During any surgical intervention, after the 24th week, cardiotocographic monitoring of the foetus should be carried out to ensure its well-being throughout the entire surgical procedure [16]. In addition, the woman must be made aware of possible complications, so consent must be obtained for an emergency caesarean section. In the case of chemotherapy treatment, however, fetal well-being is monitored after each administration. Careful planning of these treatments according to gestational age is always desirable. For example, chemotherapy treatment should be discontinued approximately three weeks before delivery, so it should not be administered later than 35 weeks [16,22]. This interval allows the excretion of drugs by the foetus through the placenta, which might otherwise persist in the infant's circulation [22].

If it were possible, the delivery should be planned and conducted exclusively on the basis of obstetrical indications, so a full-term delivery is desirable in order to avoid the consequences of a premature birth [16,22,30,31]. When preterm delivery is extremely necessary, it is important to ensure a good adaptation of the infant, e.g. by providing lung maturity through the administration of cortisone [16]. Planned preterm delivery is only justified if there is a danger to the life of the mother or the foetus [30].

Vaginal delivery is generally appropriate, but the mode of delivery is to be assessed on an individual case basis, especially depending on the type of tumour. In the case of tumours affecting the pelvic-abdominal region, such as colon cancer or gynaecological tumours in general, a caesarean section is more appropriate. In the case of cervical cancer, however, a caesarean section is recommended if the tumour is more than 3 mm deep, due to the risk of haemorrhage or obstruction of labour, or a spontaneous delivery is possible [17].

After delivery, the placenta should always be examined by histological investigation; this procedure is particularly indicated in patients with leukaemia and melanoma [16,30,31]. However, placental metastases remain a rare event [30].

2.1.4 Cancer and fertility

2.1.4.1 Cancer during reproductive age

The increase in the number of children, adolescents and young adults cancer patients is becoming a growing concern, and such patients are expected to fully recover and survive for a long time [32,33]. The overall five-year survival rate of children, adolescents and young adults diagnosed with cancer is reported to be approximately 80% [34,35]. In Italy, 5000 women of reproductive age develop cancer each year [36].

2.1.4.2 Fertility preservation and future family planning

Reproductive health is a major concern for a young woman diagnosed with cancer. The chance of having children after a cancer diagnosis is influenced by biological, psychological, and social factors [37]. Oncological treatments can adversely affect fertility [38-40]; direct effects on the female reproductive system include loss of ovarian follicles with an increased risk of infertility and premature ovarian failure [41,42]. In addition, the fear of disease recurrence may also play a role in the decision to become pregnant [43].

Today, fertility preservation techniques are used to protect and preserve the reproductive potential of individuals undergoing cancer treatment, and these strategies are critical for patients who may be at risk of infertility due to the adverse effects of cancer therapies such as chemotherapy, radiation and surgery on their reproductive organs and functions.

Several fertility preservation options are available, including cryopreservation of oocytes (retrieved, frozen, and stored for later fertilization and implantation) and/or cryopreservation of embryos (oocytes are retrieved, fertilized in vitro to obtain embryos, and then frozen for future use). Cryopreservation of ovarian tissue may also be considered: tissue containing immature eggs is surgically removed and frozen for reimplantation or in vitro maturation. Recent advances have shown a successful pregnancy rate of around 33% with this method [44]. Ovarian transplantation, or oophoropexy, may also be considered to preserve a woman's fertility by preventing damage to the ovaries during radiotherapy [45]. Using eggs from a donor for ART is an option if the patient becomes infertile after cancer treatment.

Fertility preservation should be discussed and considered early in the treatment planning phase for oncology patients to ensure the best possible outcomes for their future reproductive desires. The choice of method depends on the individual's age, type of cancer, treatment plan, and personal preferences. It's essential for healthcare providers to work closely with reproductive specialists to provide comprehensive information and guidance to patients facing fertility preservation decisions. It is important to have an individualized discussion about fertility. The desire to have a family and the number of children desired may vary depending on the age, life course parity, and lifestyle of the oncological patient [46].

2.1.5 Administrative data: potential and limitations

In the field of cancer research, administrative data can be used to study several aspects. From an epidemiological perspective, administrative data can be analysed to assess cancer incidence, prevalence, geographical distribution and trends over time, providing valuable information for planning public health. Researchers can evaluate the effectiveness of different therapies and identify areas for improvement by examining the types of treatment cancer patients receive and their adherence to guidelines. Administrative data can also help with prognosis and patient counselling by allowing analysis of long-term outcomes, including survival rates and prognostic factors associated with different types of cancer. Finally, in line with their nature, the analysis of administrative data contributes to the assessment of health care utilisation, health care costs, and resource allocation in relation to cancer care.

However, the use of administrative data in cancer research has certain limitations. First, this type of data often lack detailed clinical information, limiting the ability to delve into specific clinical aspects of cancer, such as tumor characteristics, stage, or treatment response. In addition, data may not always be accurate or complete.

Cancer registries in Italy are specialized structures or organizations that systematically collect, store, and analyze information about individuals diagnosed with cancer. The purpose of these registries is to gather comprehensive and accurate data on cancer incidence, prevalence, and outcomes within a specific geographic area, such as a region or country. The data collected by cancer registries typically includes details about the type and stage of cancer, demographic information, treatments received, and outcomes. This information is crucial for monitoring trends in cancer incidence, assessing the impact of various factors on cancer occurrence and treatment outcomes, and facilitating research to improve cancer prevention, detection, and treatment strategies. These registries play a crucial role in understanding the burden of cancer within the population and supporting public health efforts to combat and manage cancer effectively. They contribute to evidence-based decision-making, resource allocation, and the development of targeted cancer control and prevention programs.

An ideal study should be able to link administrative data with data from the cancer registry, which includes all the detailed clinical information that is missing in the healthcare databases.

2.2 Literature review of assisted reproductive technologies

2.2.1 Infertility: definition and numbers

The World Health Organisation (WHO) defines infertility as a disease characterised by the failure to achieve pregnancy after 12/24 months of unprotected, intended sexual intercourse [47]. Infertility can be either primary or secondary. Primary infertility is when a woman has never become pregnant, and secondary infertility is when she has had at least one previous pregnancy.

It is estimated that around one in six people of reproductive age around the world will experience infertility at some point in their lives. In particular, according to various estimates, infertility is relatively common, affecting about 15% of Italian couples [47]. Infertility, by definition, is not absolute; it can be treated and can have different causes, affecting women, men, or both. Couples with infertility problems can also choose to go down the path of assisted reproductive technologies (ART), which includes all the procedures available to help infertile couples achieve artificial conception. The first successful in vitro fertilisation (IVF) treatment in humans was carried out in the UK in 1978. The use of these techniques has increased steadily in recent years. In recent years, the use of ART has shown a trend of continuous growth.

According to the latest global report by the International Committee for the Monitoring of Assisted Reproductive Technologies, more than 1.9 million ART cycles are performed each year [48]. In line with the international trend, there has also been an increase in the use of these techniques in Italy, with an increase in the number of cycles per million women, reaching 7697 in 2019, albeit with large regional variations. In total, 3.1% of the country's births are ART-mediated, corresponding to over 12,000 babies born in ART clinics [49].

Table 3. Use of assisted reproductive technologies (ART) in selected countries.

Year	Italy	France	Spain	UK	Norway	Sweden	Finland	Denmark
2000		1.4		1.1	2.1	2.5	2.3	3.7
2002		1.5		1.4	2.4	2.8	2.9	4.2
2004		1.7		1.6	2.8	2.9	2.9	4.2
2006					2.8	3.3	3.3	4.1
2008	1.3					3.3	3.1	4.6
2010	1.7	2.0	2.8	2.2	4.1	3.5	3.0	5.9
2012	1.8	2.1		2.2		3.8	3.1	6.1
2014	2.2	2.3	6.4				3.1	6.4

Sources: [50-60]

2.2.2 Causes of infertility

2.2.2.1 Female causes

In the female reproductive system, infertility can be the result of a number of factors, including woman's age [36].
Anatomical abnormalities of the reproductive tract can affect fertility. Tuboperitoneal abnormalities, such as blocked fallopian tubes, can be caused by untreated sexually transmitted infections or complications of unsafe abortion, postpartum sepsis or abdominal/pelvic surgery. Abnormalities of the uterus can be inflammatory (endometriosis), congenital (septate uterus) or benign (fibroids distorting the uterine cavity). Polycystic ovary syndrome and other follicular disorders are examples of ovarian disorders.
Reproductive hormone imbalances can also be caused by disorders of the endocrine system, including the hypothalamus and pituitary glands. Examples of common disorders affecting this system include pituitary cancer and hypopituitarism.
The relative relevance of these causes of female infertility may vary from country to country, for example because of differences in the background prevalence of sexually transmitted diseases or differences in the age of the populations studied.

2.2.2.2 Male causes

Abnormalities of the male reproductive system can also cause infertility [36].
An obstruction in the reproductive tract can lead to issues with ejaculation. This blockage can arise within the sperm transportation pathways, including the ejaculatory tubes and seminal vesicles. Frequently, injuries or infections of the genital tract are the underlying causes of such blockages.
Testicular failure to produce sperm, often caused by factors like varicoceles or medical treatments that damage sperm-producing cells (such as chemotherapy), can be a contributing factor.
Also abnormal sperm function and poor quality can significantly impact fertility. Conditions or circumstances leading to abnormal sperm shape (morphology) and movement (motility) can negatively affect fertility. For instance, the use of anabolic steroids can result in atypical semen parameters, including sperm count and morphology.
Fertility can be also affected by lifestyle factors such as smoking, excessive alcohol consumption and obesity.
Finally, exposure to environmental pollutants and toxins can have a direct toxic effect on the germ cells, both eggs and sperm, resulting in a reduction in the number and poor quality of these cells.

2.2.3 Techniques: an overview

According to the American Centre for Disease Control, ART is any fertility-related treatment that involves the manipulation of eggs or embryos. For this reason, first-stage procedures in which ovarian stimulation is performed without egg retrieval and in which only the sperm is manipulated, such as intrauterine insemination, do not really fall under this definition.

2.2.3.1 Conventional in vitro fertilization versus intracytoplasmatic sperm injection

In vitro fertilization (IVF) is a well-established procedure that involves the fertilization of an egg with sperm outside the woman's body, in a laboratory setting, including several steps such as ovarian stimulation, oocyte retrieval, fertilization, embryo culture, and embryo transfer [61].
In the first procedures performed, a single oocyte was retrievied from a natural menstrual cycle; then, controlled stimulation of ovaries was used to maximize the number of oocytes gained per cycle. Selective estrogen receptor modulators, such as clomiphene citrate and tamoxifen, are the main medications used. Also injection of exogenous gonadotropins, i.e. follicle-stimulating hormone and luteinizing hormone, is commonly used in ovarian stimulation.
Subsequently, with transvaginal ultrasound monitoring, the number and growth of follicles are monitored. In the first procedures, oocyte retrieval was performed via laparotomy and then laparoscopy [62]. Today, the procedure involves transvaginal needle insertion guided by ultrasound to aspirate follicular fluid containing oocytes, typically performed with anesthesia or sedation [63]. Only in a few cases, in patients with vaginal agenesis or ovarian trasposition, laparoscopy is still necessary [61]. Spermatozoa are usually obtained by ejaculation, or surgically retrieved if azoospermia is present, and isolated by centrifugation and washing in culture media.
In a conventional IVF, the retrieved eggs are combined with sperm in a culture dish and the resulting embryos are carefully monitored and cultured in the laboratory for a few days, before one or more are selected for transfer into the woman's uterus [61].
Intracytoplasmic sperm injection (ICSI) is a particular form of IVF in which a single sperm, chosen by embryologists according to morphologic parameters, is injected directly into the cytoplasm of the oocyte to facilitate fertilization. This technique allows a more precise control of the fertilization process, increasing the chances of successful embryo development and subsequent implantation in the uterus using a catheter [61].
The choice between these techniques depends on various factors, including the specific fertility issues, the quality of the sperm, and the recommendations of the fertility specialists. ICSI is particularly beneficial for couples with a hystory of fertilization failure or with male infertility problems, such as low sperm count or poor sperm motility [64].

2.2.3.2 Preimplantation genetic testing

According to European Society of Human Reproduction and Embryology (ESHRE), preimplantation genetic testing (PGT) is a test performed to analyze the DNA from oocytes or embryos before transfer to identify possible genetic diseases [65].
PGT-A is used to determine whether the embryo has the normal number of chromosomes it needs. An embryo can have too few or too many chromosomes if the sperm or egg cells divide unequally. Aneuploidy is a major cause of failed implantation in pregnancy and miscarriage, and a major cause of birth defects in children; only one type of monosomy, Turner syndrome, which means that one of the X chromosomes is missing, can survive. Instead, trisomies of pairs of chromosomes which can results in live births are trisomy 21 (Down's syndrome), 18 (Turner's syndrome), and 13 (Patau's syndrome).
PGT-M analyses for specific gene mutations that one (or both) of the parents is known to carry, such as muscular dystrophy, cystic fibrosis, BRCA1 and BRCA2 mutations, and fragile X syndrome.
PGT-SR screens embryos from patients with known structural chromosomal abnormalities, such as inverted or translocated chromosomes. In patients with a known structural rearrangement, there is a greater risk of producing embryos that do not have the correct amount of chromosomal material and are less likely to result in a live birth. Repeated miscarriages are common in patients with these problems.

2.2.3.3 Fresh versus frozen embryo transfer

In fresh embryo transfer, a fertilised embryo is transferred into the uterus within three to five days of retrieval. In frozen embryo transfer, the embryos are frozen and implantation can take place weeks, months, or even years after oocytes collection and fertilisation.
Pregnancies after fresh embryo transfer can develop ovarian hyperstimulation syndrome, a potentially life-threatening iatrogenic complication. This syndrome is characterised by a shift of fluid from the blood vessels into the abdominal cavity, which can lead to abdominal distension, a high risk of thrombosis and reduced blood supply to vital organs such as the kidneys and liver [66].
In order to reduce ovarian hyperstimulation syndrome by avoiding fresh embryo transfer, the option of a "freeze-all" strategy has been considered. All suitable embryos are frozen after IVF/ICSI treatment and only cryopreserved embryos are transferred in subsequent cycles [67].
Recent technical advances in cryopreservation have improved the chances of embryo survival after thawing, resulting in higher pregnancy rates [48].
Between the "freeze all" strategy and the conventional IVF/ICSI strategy, there is probably little or no difference in the cumulative live birth rate and the clinical pregnancy rate. A recent Cochrane review suggested that the cumulative live birth rate is 58% with a conventional IVF/ICSI strategy and between 57% and 63% with a "freeze-all" strategy.[68].

2.2.4 The challenges of twin pregnancies

In order to maximize pregnancy rates, the transfer of multiple embryos has historically been the practice in IVF. This increases the risk of multiple births. Multiple births are known to be at significant risk for a number of adverse outcomes, including preterm birth, low birth weight, congenital malformations, perinatal mortality, and long-term morbidity and disability in survivors. As the ultimate goal of ART is the birth of a healthy infant, reducing or eliminating multiple pregnancies must be an important consideration.

In recent years, the proportion of multiple births among ART babies has continued to fall as a result of changes in approach [69]. In particular, in order to reduce the multiple birth rate associated with ART, a strategy promoting elective single embryo transfer combined with frozen embryo transfer was adopted [70,71]. A meta-analysis has shown that the odds of having a multiple live birth were significantly lower in women who were randomised to elective single embryo transfer than in women who received a double embryo transfer [72].

According to data from the Italian ART registry [49], as regards the distribution of transfers by number of embryos transferred, the percentage of transfers with one embryo is increasing for all the techniques used (+2.7% for fresh embryos, +3.4% for frozen embryos and +3.5% for frozen oocytes). At the same time, transfers with two embryos (-0.9%, -3.0% and -3.6% respectively) and with three embryos are decreasing (-1.5% and -0.3% respectively), except for the frozen oocyte technique (+0.1%).

Indeed, other ART procedures (such as prolonged culture and late stage transfer) may be associated with multiple pregnancies and spontaneous zygote cleavage may occur after transfer but before implantation [73].

2.2.5 Advantages and disadvantages of administrative data

Data on ART reported in CedAP database were not always accurate and were reported directly by women. It is specified whether the conception was spontaneous or after assisted reproduction and, if applicable, the type of technique chosen. In particular, medically assisted reproduction includes techniques of the first stage - pharmacological ovulation induction or IUI - ICSI or conventional IVF.

The register of outpatient services can also be used to track procedures such as echo-guided needle aspiration of follicles, artificial insemination, sperm capacitation, and ovarian ultrasound (to monitor ovulation). In addition, in the pharmaceutical prescription register, it is possible to follow the prescription of drugs belonging to the class of gonadotropins and other ovulation simulators.

However, information regarding laboratory procedures (embryonic biopsy, assisted hatching, number of embryos transferred) are not reported in the administrative databases. Moreover, it is not possible to determine whether the embryo transfer is carried out with a frozen or fresh embryo, or whether the women had recourse to oocyte donation.

On the other hand, the Italian ART registry contains all these details. However, the data currently available is in an aggregated format, meaning that it has been combined and summarised. The specific details of each woman's case are not available. This aggregation process consolidates the data into general trends, statistics or summaries, making it impossible to carry out research or analysis that looks at each individual woman receiving ART.

2.3 Literature review of pharmacoepidemiology in pregnancy and during postpartum

2.3.1 Use of drug in pregnancy

2.3.1.1 Pharmacoepidemiology: trends and patterns in medication utilization

Pharmacoepidemiology in pregnancy is an important subdiscipline at the interface of pharmacology, epidemiology and maternal-fetal medicine. It deals with the complex study of medication use during pregnancy, seeking to understand its impact on both maternal and fetal health. The unique physiological changes that occur during pregnancy often require careful consideration when prescribing medications to expectant mothers, making pharmacoepidemiology an essential discipline in modern healthcare. This multifaceted field not only examines the prevalence and patterns of medication use in pregnancy, but also examines the potential risks and benefits associated with different drug exposures. Understanding the rational use of medications and exploring patterns of use is crucial, and monitoring prescription patterns during pregnancy could be an extremely important tool for assessing compliance with recommended supplement and medication use in clinical practice.
The use of medicines during pregnancy is very common, both nationally and internationally. Information on the benefit-safety profile and potential teratogenic effects of some medications during pregnancy remains uncertain. Updating the knowledge of health professionals and communicating the risk to women are complex and underestimated issues. Population-based studies on drug use during pregnancy in Italy are scarce and not recent.
According to evidence [74], high-income countries have high rates of prescription drug use during pregnancy, with overall estimates ranging from 27 % to 99 % of pregnant women filling at least some prescription, including vitamins and minerals. It is difficult to compare the results of studies on medication use because of differences in study methods. Looking only at studies using the outpatient prescription register, the lowest rates of prescription drug use during pregnancy were reported in Northern Europe (44-57%), while the highest rates were observed in Germany (85%) and France (93%). In Italy, in 1986, more than 2000 women giving birth in the city of Turin were interviewed and more than 80% of them reported having taken at least one drug during pregnancy, with an average of 4 drugs per user; this study denounced the tendency towards overmedication and the persistence of outdated practices [75]. In the same years (1989-1992), about 55% of women in Emilia-Romagna and Tuscany used at least one drug during pregnancy [76]. At the end of the 1990s, a more comprehensive survey of over 9000 women from 13 Italian regions found that 75% had used at least one drug during pregnancy, with the proportion of use increasing over time from around 30% in the first trimester to 51% in the third trimester [77]. All the studies cited are interview-based, so a potential bias must be taken into account, as women do not

always disclose the use of all drugs during pregnancy [78]. More recently, automated databases have been used to study medication use in pregnancy, providing detailed prescription information collected prospectively for large cohorts of pregnant women from the regions of Emilia-Romagna [78,79], Lazio [79-81] and Puglia [79]. Recently, for the first time, the Italian Medicines Agency (AIFA) published a report based on the linkage of different regional health information streams, which made it possible to identify which drugs are prescribed to pregnant women in Italy and for which clinical conditions [82].

2.3.1.2 Pregnant women in clinical trials: ethical considerations and implications for drug development

Historically, pregnant women have been systematically excluded from participation in clinical trials, leading to a substantial void in our understanding of the safety, effectiveness, and appropriate dosing of prescription medications employed during pregnancy. This persistent exclusionary practice has contributed to a critical deficit in evidence-based guidance for healthcare professionals and expectant mothers.

In the context of pre-authorization research of new medications, pregnant and lactating women are considered *special* and *vulnerable*. The risks of therapeutic research in pregnancy should be weighed against the risks in the clinical domain of giving a drug when dosing information and efficacy/safety profile in pregnancy are unknown or with holding a potentially beneficial treatment because of lack of evidence [83,84]. As a result, most available drugs were not adequately studied despite several physiological, pharmacokinetic and pharmacodynamic changes occurring during pregnancy. Therefore, when faced with the delicate task of managing conditions or administering treatments during pregnancy, healthcare providers often face a difficult decision marked by uncertainty.

In the United States, the 21st Century Cures Act established a task force on Research Specific to Pregnant Women and Lactating Women to advise the Secretary of Health and Human Services (HHS) regarding gaps in knowledge and research on safe and effective therapies for pregnant women and lactating women [85]. The key purpose of this task force was to develop "*a plan to identify and address gaps inknowledge and research regarding safe and effective therapies for pregnant women and lactating women, including the development of such therapies*" (21st Century Cures Act, Pub. L. No. 1072, 114-255§2041; 2016).

The thalidomide scandal of the late 1950s and early 1960s remains an indelible stain on the history of pharmaceuticals. Thalidomide was initially marketed as a seemingly harmless sedative and antinausea drug and was widely used, particularly by pregnant women, to alleviate morning sickness. Tragically, the use of thalidomide during pregnancy was found to be associated with severe birth defects, leading to a generation of children born with limb deformities and other devastating health problems. This shocking revelation exposed the profound lack of adequate drug testing and regulatory

oversight at the time, and highlighted the urgent need for stronger safeguards in the pharmaceutical industry. The Thalidomide tragedy ultimately led to significant changes in the drug approval process and increased scrutiny of drug safety, culminating in more rigorous clinical trials and regulatory oversight to prevent such catastrophic events from happening again.

Frances Kelsey, a well-known pharmacologist and physician, played a key role in preventing the approval and distribution of Thalidomide in the United States during the 1960s. Revisiting her principles, which emphasised the importance of remaining vigilant, putting safety first and minimising the risks associated with medicines, serves to advance our ethical responsibility to protect pregnant people through thorough research. It will also help generate pregnancy-specific data for evidence-based healthcare, while maintaining Kelsey's commitment to protecting pregnant women and their children from the potential dangers of untested medicines [86].

2.3.1.3 Pharmacokinetics

Understanding the dynamics of drug absorption, distribution, metabolism, and excretion during pregnancy is of paramount importance in the field of pharmacokinetics. Pregnancy induces several physiological changes in a woman's body that can significantly affect the pharmacokinetics of drugs administered during this period. These modifications can affect both the efficacy and safety of pharmaceutical interventions by influencing drug concentrations in both the maternal and fetal compartments. Following, the complex interplay between pharmacokinetics and pregnancy was approached, sheds light on how these physiological changes may affect drug disposition, and provides insights into the challenges and considerations for healthcare professionals when managing medication regimens in pregnant women.

First, the drug absorption is affected by the reduction in intestinal motility, prolongation of gastric and intestinal emptying time, and reduction in gastric acid secretion and increase in mucus secretions. This may be important in clinical situations where the drug needs to act quickly [14]. In addition, nause and vomiting, experienced by up to 80% of women during the first three months of pregnancy [87], can result in low plasma drug concentrations due to drug loss during vomiting. This is important when deciding whether to repeat a dose or wait for the next dose.

Second, the distribution of drugs may be influenced by a significant expansion of plasma volume by approximately 50% [88]. These changes can lead to variations in drug concentrations within maternal compartments and, consequently, may affect both the efficacy and safety profiles of pharmaceutical agents administered during pregnancy.

Third, during pregnancy, substantial alterations in renal and hepatic function can profoundly impact the process of drug elimination within the maternal body. These changes include increased renal blood flow and glomerular filtration rate, potentially leading to enhanced renal excretion of drugs, as well as variations in hepatic enzyme

activity, which can affect drug metabolism and clearance. Consequently, careful consideration of these physiological shifts is essential when managing medication regimens for pregnant individuals to ensure both maternal and fetal safety.
An important issue in pharmacotherapy during pregnancy is the transport of drugs across the placenta. Although the placenta acts as a barrier between the maternal and fetal circulations, it allows the transfer of certain drugs, creating the potential for fetal exposure to maternal drugs. Particularly, a number of compounds are capable of passive diffusion across the placenta, particularly those that are small (<500 Da) and non-polar [89]. A few years after the discovery of the thalidomide scandal, the Slone Epidemiology Center Birth Defects Study at Boston University began conducting a series of detailed interviews with a diverse selection of pregnant women with the intention of monitoring the medications these women were taking [90]. Once the data was collected, the researchers analysed the trends in medication use in pregnancy and compare the increasing use with the causes and occurrence of birth defects. This raises critical concerns about the safety of pharmaceutical interventions during pregnancy. In particular, the teratogenic potential of drugs adds complexity to these considerations and warrants thorough risk assessment and informed decision-making regarding the use of drugs during pregnancy.
In the context of these intricate pharmacokinetic changes, healthcare providers are faced with the challenge of personalising medication management for pregnant patients. Close monitoring of drug levels, vigilant dosage adjustments, and understanding of the evolving maternal physiology are essential to ensure optimal therapeutic outcomes while safeguarding the health and well-being of both the expectant mother and the developing fetus.

2.3.1.4 Teratogenic classification

The teratogenicity remains a central concern in pharmacology and maternal-fetal medicine. A drug is considered teratogenic if it causes, directly or indirectly, a structural or functional abnormalities in the fetus or child when administered to the pregnant mother. Since organogenesis takes place during the first 3 months of pregnancy, teratogenic drugs taken during this period tend to cause structural abnormalities. After the first trimester, teratogenic drugs may cause growth defects.
Understanding the mechanisms, factors and classifications associated with drug teratogenicity is essential to guide clinicians in safely prescribing drugs to pregnant women and protecting the well-being of the developing fetus. Women need to be aware of the medications they are taking if they are planning a pregnancy, so that if any of the medications are potentially teratogenic, they can either be stopped, changed, or reduced to the lowest possible dose. When stopping a drug, the risk to the foetus must always be weighed against the risk to the woman.

In 1979, the Food and Drug Administration (FDA), the United States federal agency of the Department of Health and Human Services, established five risk

categories to indicate the potential for a drug to cause birth defects when used during pregnancy. The categories are the result of an assessment of the reliability of the data from animal and human studies and the risk-benefit ratio of the drug [91]. Such classification system provides a therapeutic guidance for the clinician (**Table 4**).

Table 4. Food and Drug Administration (FDA) classification.

Category A	Adequate and well-controlled studies have shown no risk to the fetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters).
Category B	Animal studies have revealed no evidence of harm to the fetus, but there are no adequate and well-controlled studies in pregnant women, or animal studies have shown an adverse effect that was not confirmed in human studies.
Category C	Animal studies have shown adverse effects on the fetus, and there are no adequate and well-controlled studies in humans, but the potential benefits may outweigh the risks.
Category D	There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved may outweigh potential benefits.
Category X	Studies in animals or humans have demonstrated fetal abnormalities, and the risks clearly outweigh any potential benefits. These drugs should be avoided during pregnancy.

Source: [91]

An alternative classification was proposed by the Australian Drug Evaluation Committee (ADEC) [92]. This committee introduced its own classification system to assess the safety of medications during pregnancy (**Table 5**).

Table 5. Australian Drug Evaluation Committee (ADEC) classification.

A	Drugs which have been taken by many pregnant women and women of childbearing age without an increase in the frequency of malformations or other direct or indirect harmful effects on the fetus having been observed.
B1	Drugs which have been taken by only a limited number of pregnant women and women of childbearing age, without an increase in the frequency of malformation or other direct or indirect harmful effects on the human fetus having been observed. Studies in animals have not shown evidence of an increased occurrence of fetal damage.
B2	Drugs which have been taken by only a limited number of pregnant women and women of childbearing age, without an increase in the frequency of malformation or other direct or indirect harmful effects on the human fetus having been observed. Studies in animals are inadequate or may be lacking, but available data show no evidence of an increased occurrence of fetal damage.
B3	Drugs which have been taken by only a limited number of pregnant women and women of childbearing age, without an increase in the frequency of

	malformation or other direct or indirect harmful effects on the human fetus having been observed. Studies in animals have shown evidence of an increased occurrence of fetal damage, the significance of which is considered uncertain in humans.
C	Drugs which, owing to their pharmaceutical effects, have caused or may be suspected of causing, harmful effects on the human fetus or neonate without causing malformations. These effects may be reversible.
D	Drugs which have caused, are suspected to have caused or may be expected to cause, an increased incidence of human fetal malformations or irreversible damage. These drugs may also have adverse pharmacological effects.
X	Drugs which have such a high risk of causing permanent damage to the fetus that they should not be used in pregnancy or when there is a possibility of pregnancy.

Source: [92]

A study comparing these two classification systems and another from Sweden found differences in the assignment of drugs to different risk factor categories and highlighted the need for a single indicator that includes several factors that make the use of a drug during pregnancy safe or unsafe, such as dose, time and duration of exposure, and comorbidity [93].

2.3.2 Use of drugs during lactation

2.3.2.1 Drug transfer to breast milk and risk of infant toxicity

Over half of women take medication while breastfeeding, exposing their infants to medication through breastfeeding [94,95]. Despite adverse drug reactions may occurr in the infant [96]; according to several guidelines, only a small percentage of drugs have contraindications during lactation [97-99].

However, healthcare providers tend to advise mothers not to breastfeed while taking medication [100,101] because of the limited data on medication during lactation, but also because of the challenge of disseminating information on the safety of medication use during certain periods.

Infant exposure to maternal drugs in breast milk is less than 10% of the exposure level experienced by the fetus in utero for most drugs [102]. Several factors explain the relatively low drug exposure during lactation [96]. First, the amount of drug excreted in breast milk is relatively low throughout the lactation period. This is because the mammary gland does not act as a primary drug excretory organ like the kidney. The concentration of a drug in milk depends from maternal plasma concentration and plasma protein binding, size of the drug molecul, degree of ionisation, and lipid solubility [103]. Factors such as maternal dose and clearance are critical in determining fetal exposure during pregnancy [96]. In contrast, for infant exposure via breast milk, factors include the infant's dose from the milk and the infant's clearance of the drug. The infant's dose from milk is generally small, being an order of magnitude or more lower than the maternal dose and much less than the known safe doses of the drugs used in neonates and infants. Secondly, during the first 2-3 days of an infant's life, milk intake is minimal. Drug clearance in infants tends to increase as their milk intake increases. However, during the first days when infant's drug clearance is lower, the limited intake of maternal milk compensates.

Practice points for prescribing in breastfeeding was recently proposed [103]: prescribing the lowest effective dose, if necessary, temporarily interrupting breastfeeding, feeding the baby just before the mother takes a medicine to ensure that the baby receives the lowest possible concentration of the medicine, choosing alternative routes or products to minimise systemic exposure in the mother, and choosing medicines with a relatively short half-life.

Information on drugs and other chemicals to which breastfeeding mothers may be exposed is contained in the LactMed® database [104]. It provides information on the levels of these substances in breast milk and in the blood of the infant, and on the possible adverse effects in the infant. Where appropriate, therapeutic alternatives to these drugs are suggested. All data are derived from the scientific literature and are fully referenced. Data are reviewed for scientific validity and currency by a peer review panel.

2.3.2.2 A focus on antidepressant drugs

Mothers may need medication not only for chronic conditions resistant to pregnancy, but also for acute conditions, in some cases related to pregnancy itself. For example, in the postnatal period, mothers may experience mental and emotional disorders of varying types and severity, such as the maternity blues, postpartum depression or phsycosis.

Antidepressant pharmacotherapy is indicated for moderate to severe depression or when it has failed to respond to initial psychotherapy. With an estimated international prescribing prevalence of 4.7% in the first year postpartum, selective serotonin reuptake inhibitors are the mainstay of pharmacological treatment for perinatal depression [105]. Managing antidepressant treatment in the postpartum period, particularly during breastfeeding, is complex and must balance the potential risks of exposing the infant to these drugs that pass into breast milk with the potential adverse effects of poorly managed peripartum maternal depression on both mother and child. Fluvoxamine, paroxetine, and sertraline are preferred in breastfeeding women leading to the lowest serum medication levels in infants [106]. Even today, many European countries do not follow fully consistent and updated clinical practice guidelines regarding pharmacological treatment of peripartum depression [107].

According to a study carried out in the USA, about half of the women who were diagnosed with postpartum depression were taking an antidepressant [108]. It's important to note that the way in which postpartum depression is treated varies between areas. For example, the use of antidepressants tends to be lower in European countries than in the USA [105]. This discrepancy, possibly due to differences in medical practice and cultural norms, raises awareness of the potential undertreatment of women with new-onset postpartum depression in some countries.

2.3.3 Use of administrative data for pharmacoepidemiological studies

Clinical trials before drugs are approved for marketing are limited to the patients enrolled in the trial and may provide limited safety data, so it is necessary to study the real-world use of drugs in clinical practice. In addition, because pregnant and breastfeeding women are considered *special* and *vulnerable* in the context of pre-authorisation research for new medicines, and because initial clinical trials in drug development generally exclude women in this phase of their lives, they do not provide sufficient evidence of efficacy and safety in this specific population.

Pharmacoepidemiology is the study of how medications interact with real-life conditions, risks and benefits. The main aim of this discipline is the rational use of medicines.

Pharmacoepidemiological databases could be used for several purposes, such as drug use, drug effects and monitoring programmes to improve drug use [109]. As for drug use, in Italy, automated databases have been used to investigate the drug utilization in pregnancy providing detailed prescription information collected prospectively for large cohorts of pregnant women from Emilia-Romagna [78,79], Lazio [79-81], and Puglia [79] regions. In 2020, for the first time, the Italian Medicines Agency (AIFA) published a report based on the linkage record of different regional health information flows that has made it possible to detect which drugs are prescribed to pregnant women in Italy and for which clinical conditions [82]. With regard to drug effects, several studies have been carried out using the same databases as those used for the investigations presented in this thesis to assess whether the use of selected drugs, such as antidepressants [110], antibiotics [111], or antihypertensive drugs [112], is associated with adverse neonatal outcomes.

Data from medical record databases relate to prescriptions and are recorded as part of the process of clinical outpatient care. They reflect the physician's point of view. Administrative data relate to dispensed medications and are obtained from pharmacy billing and used for other fiscal functions. As some prescriptions written by doctors are never filled at the pharmacy, it might be expected that dispensing records would more closely reflect actual drug use than prescribing. However, not all dispensed doses are actually used, so the dispensing data overestimate exposure. In addition, information on smoking habits, alcohol consumption and body mass index are missing in the administrative databases.

Some biases should be considered; confounding by indication, unmeasured/residual confounding, outcome misclassification, and immortal time bias are the most important [113]. When the completeness or quality of the original data is in question, linking medical records to administrative databases can be useful to minimise the risk of bias and to validate administrative data with primary data collected using ad hoc methods. Specifically for pregnancy, one study [114] provides an overview of the limitations of studies using linked administrative data. First, dose, duration and timing of exposure are not consistently addressed. Second, there is a great deal of variability in the way in which drug exposures are classified and in the way in which women who

stop taking their drugs before or during the first trimester are treated in the analyses. Finally, there are concerns in making assumptions about how and when women who receive a prescription for a medication actually take it during pregnancy.

CHAPTER 3 - EXPERIMENTAL SECTION: SOURCES & METHODS

3.1 Sources

The administrative databases on health care utilization given by Lombardy, the largest region in Italy with a population of over 10 million, provided the data used for the investigations reported in this thesis. At the national and regional levels, Italy has set up a number of reliable systems for gathering epidemiological data. The National Health Service (NHS), a system that provides universal healthcare coverage and is financed by taxes, was established by the Italian government in 1978. This system ensures accessibility for the whole population by providing free or subsidized healthcare services to all Italian citizens and residents. Decentralized to the regional level, the NHS functions under the Ministry of Health's jurisdiction. Each region has exclusive powers to design and manage health services and to set the funding parameters for local health authorities and hospitals. Lombardy has been using an automated database system with a vast amount of data since 1997.

Administrative databases on health care utilisation are large repositories of data on health care systems that are routinely collected by health care providers and other institutions; for example, through a visit to a doctor's office, a diagnostic procedure, an admission to hospital, or a prescription filled at a community pharmacy. Administrative data are typically collected for purposes such as payment and reimbursement. It is therefore reliable and useful for researchers to understand the incidence of disease and side effects, and to assess and improve the quality of health care. However, as these databases are not designed for clinical research, the clinical content of the administrative data may only include the demographic characteristics and diagnoses of the patients and the medical codes for the procedures performed, and may not be accurately captured. They should therefore be used with caution for research purposes.

For the purposes of the projects carried out, data were obtained from the following databases: i) an archive of beneficiaries of the regional health service, ii) the hospital discharge register, iii) the outpatient drug prescribing register, iv) the outpatient specialistic visits register, and v) the certificates of delivery assistance (CeDAP) database. Below is a brief description of each.
The archive of beneficiaries of the regional health service includes the entire resident population, with demographic and administrative data, such as sex, date of birth, date of start and end of assistance, place of residence, and, if applicable, date of death.
The hospital discharge (scheda di dimissione ospedaliera – SDO) registry was established by decree of the Ministry of Health on 28 December 1991 as a standard tool for collecting information on each patient discharged from public and private hospitalisation facilities throughout the national territory. The information collected includes personal details on the characteristics of the hospitalisation (e.g. institution, hospital department, date of admission and of discharge, hospitalisation-related costs coded according to the national Diagnosis Related Group system – DRG) and clinical

characteristics (e.g. principal diagnosis, concomitant diagnoses, date and type of diagnostic or therapeutic procedures; coded according to the International Classification of Diseases, Ninth Revision - Clinical Modification – ICD-9-CM). Information on drugs administered during hospitalisation or adverse reactions to them (subject to other specific information flows) is excluded from the discharge form.

The database on drug prescriptions covers all outpatient drug prescriptions reimbursed by the national health system and dispensed by pharmacies. The database on drug prescriptions covers all outpatient drug prescriptions reimbursed by the national health system and dispensed by pharmacies, including information such as the specific drug according to the Anatomical Therapeutic Chemical (ATC) coding system, the quantity and the cost. Prescriptions of innovative and expensive drugs administered in hospital on an outpatient basis or distributed for home therapies are contained in another registry: File F.

The database on outpatient specialist and diagnostic services provides information such as patient characteristics, health facility, type of service, and amounts. Emergency department visits not followed by hospitalisation are included.

The CedAP, established by Decree of the Minister of Health 16 July 2001, n. 349, is the richest source of health, epidemiological and socio-demographic information on births at national level and is an essential tool for for planning and supporting public health policies related to childbirth at national and regional levels. At the time of delivery, midwives complete this form, which includes a unique anonymous identification code for the mother and one for the newborn, personal details and socio-demographic characteristics of both parents (e.g. level of education, employment), detailed information on the type of pregnancy (single or multiple), mode of conception (natural or assisted), course of pregnancy (physiological or not), mode of labour, mode and timing of delivery, maternal outcomes and newborn health (e.g. birth weight, Apgar score).

3.2 Study design and main methods

The projects presented focus on the design of historical cohort studies. These studies are not only historical in nature, but also population-based, providing a comprehensive perspective on the conditions and outcomes under investigation. The following sections explore the complexity of these cohort studies, examining their methodologies and analytical approaches.

3.2.1 Record linkage

The information collected in each of the above databases is anonymised through the use of a unique identification number assigned to each NHS beneficiary. Thus, the different databases can be linked to each other with record linkage technique, an algorithmic procedure whose purpose is to identify and merge records from one or more data sources that correspond to the same individual.
This method makes it possible to reconstruct the entire diagnostic-therapeutic process of a person, integrating the various services that he or she may have received potentially throughout his or her life, or during the entire period of his or her residence in the region.

3.2.2 Birth cohorts

The studies presented include birth cohorts identified through the record linkage between the SDO and CedAP databases. CedAP forms without a link to the mother's hospitalisation for childbirth were excluded.
The general inclusion criteria are given above, while the specific or modified criteria for each study are given in the relevant chapter.

a) Births of women aged 15 to 55 years;
b) Births between the 22nd and 42nd gestational week;
c) Births for which the linkage between mother and newborn was possible;
d) Births without missing (or not clear information) regarding the exposure (study specific);
e) Births without missing (or not clear information) regarding the outcome (study specific);
f) Births of women resident in Lombardy and covered by NHS for the whole period of follow-up (study specific).

3.2.3 Statistical methods

The general methods underlying all studies are described below. The specific methods of the individual studies can be found in the respective chapters.

Standard descriptive statistics, including frequencies and percentages for categorical data and means and medians for quantitative data, were calculated to assess the distribution of maternal sociodemographic and clinical characteristics according to the specific exposure evaluated in each study. The Chi-square test (or its version for the trend), Fisher's test and absolute standardised differences were used when appropriate for testing differences or trends in categorical variables. Independent samples t-test was used to compare numerical variables.
Logistic or log-binomial regressions were fitted to estimate the odds ratio (OR) or prevalence ratio (PR) and 95% confidence interval (95% CI) of each outcome associated with exposure (study specific). Models were adjusted for potential confounders.
All analyses were performed using the Statistical Analysis System Software (version 9.4; SAS Institute, Cary, NC, USA). Statistical significance was set at the 0.05 level.

According to Italian law, the analysis of an anonymised administrative database does not require ethics committee approval. All data were anonymised.

3.3 Strenghts and limitations

Administrative databases have become the main source of data in many epidemiological fields over the last two decades. The advantages and disadvantages of using of this kind of data in epidemiological, clinical, and healthcare research are well known [115-118].

Notable advantages include large sample size, population coverage and generalisability, which allow researchers to effectively mirror the real-world practice. Secondly, this methodology avoids additional costs for data collection, offers extended observation periods and provides the opportunity to link various databases containing several patient information on diagnostic and therapeutic aspects. In addition, data typically remain accessible for substantial durations and are update to date, allowing for comprehensive trend analysis.

Disadvantages include the uneven quality of the data collected, in terms of completeness and accuracy, lack of homogeneity of compilation, and variation of classification systems over the years. In line of this, the information that is controlled for the purpose of reimbursement may be of a better quality than other information [119]. Furthermore, as these databases are not designed for clinical research, certain information critical to clinical research is missing, particularly details typically documented in medical records. There are also challenges in drawing causal inferences due to potential bias or confounding, and the possibility of misclassification of outcomes or exposures. Moreover, countries differ in the way they manage the financing of public and private health care institutions. Therefore, administrative databases may contain data from public and private institutions or only public institutions. In the second case, only some of the patients are covered.

The specific strengths and limitations of each study are indicated in the relevant chapters.

CHAPTER 4 - EXPERIMENTAL SECTION: PROJECTS

4.1 Pregnancy-associated cancers

4.1.1 I Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Franchi M, Dalmartello M, Scarfone G, Negri E, Parazzini F, La Vecchia C, Corrao G. Obstetric and neonatal outcomes in women with pregnancy associated cancer: a population-based study in Lombardy, Northern Italy. BMC Pregnancy Childbirth. 2021 Jan 7;21(1):31. doi: 10.1186/s12884-020-03508-4 [120]**

4.1.1.1 Specific aim and methods

This research project aims to explore the complex relationship between cancer and pregnancy, the intriguing possibility that they may coexist, and to analyse the potential impact this may have on perinatal outcomes.
The main aim of the paper was to examine how the presence of cancer might affect pregnancy, in particular assessing the association between the diagnosis of PAC and selected adverse perinatal outcomes. The management of labour and delivery was compared in women diagnosed with PAC and those without cancer to analyse the association of cancer with induction of labour and iatrogenic delivery. The timing of delivery was also taken into account. Adverse neonatal outcomes considered included small for gestational age, low Apgar scores, major birth defects, and perinatal mortality.

For this study, in a cohort of women hospitalised for childbirth between 2008 and 2017, mothers were identified as having PAC if they had at least one hospitalisation with main or secondary diagnosis of cancer during pregnancy up to one year after delivery. A secondary exposure was also taken into account: chemotherapy. The prevalence of PAC in the study cohort was computed by dividing the observed number of cases, overall and by cancer type, by the total number of deliveries.

Each woman diagnosed with PAC was matched with four randomly selected women who were cancer-free. Matching was done by maternal age and calendar year at birth.

Standard descriptive statistics, including frequencies and percentages for categorical variables and means and standard deviations for continuous variables, were calculated to assess the distribution of maternal sociodemographic and clinical characteristics according to exposure to pregnancy-related cancer. Absolute standardised differences were used to test for differences in categorical variables between the group of women diagnosed with PAC and those without. Independent samples t-tests were used to compare numerical variables between the two groups.

Log-binomial regression models were fitted to estimate the prevalence ratio (PR) and 95% confidence interval (95% CI) of adverse perinatal outcomes associated with maternal diagnosis of PAC. Models were adjusted for socio-demographic characteristics, selected comorbidities, and current obstetric history. To account for the potential correlation of women with more than one birth during the study period, generalised estimating equations were used.
All the analyses were performed among strata of timing of PAC diagnosis (i.e. during pregnancy and post pregnancy). The occurrence of perinatal outcomes was also documented individually for the three most common cancers (breast cancer, thyroid cancer and lymphoma) and compared using the Chi-square test or Fisher's exact test.

4.1.1.2 Results

In the selected cohort, 831 women diagnosed with PAC were identified, corresponding to 1.26 cases per 1000 births. As regard timing of cancer diagnosis, 30 (3.6%) cases were diagnosed in the first trimester of pregnancy, 53 (6.4%) cases in the second, and 103 (12.4%) cases in the third; while 645 (77.6%) were diagnosed during the year post-pregnancy. The most common cancer site was breast (N=259, 39.3 cases per 100,000 births), followed by thyroid (N=138, 21.0 cases per 100,000 births) and lymphoma (N=103, 15.3 cases per 100,000 births). PAC were matched to 3324 cancer-free women.

Table 6. Selected maternal characteristics of the cohort. Lombardy, 2008-2017.

	PAC women, N=831 n (%)	Cancer-free women, N=3324 n (%)	Standardized difference (absolute)
Maternal age			
< 30	107 (12.9)	428 (12.9)	Matching variable
30-34	260 (31.3)	1040 (31.3)	
35-40	379 (45.6)	1516 (45.6)	
>40	85 (10.2)	340 (10.2)	
Mean (SD)	34.8 (4.6)	34.8 (4.6)	
Calendar year at birth			
2008	84 (10.1)	336 (10.1)	Matching variable
2009	75 (9.0)	300 (9.0)	
2010	100 (12.0)	400 (12.0)	
2011	98 (11.8)	392 (11.8)	
2012	83 (10.0)	332 (10.0)	
2013	82 (9.9)	328 (9.9)	
2014	87 (10.5)	348 (10.5)	
2015	84 (10.1)	336 (10.1)	
2016	86 (10.4)	344 (10.4)	
2017	52 (6.3)	208 (6.3)	
Nationality			

Italian	725 (87.2)	2808 (84.5)	0.078
Foreign	106 (12.8)	516 (15.5)	-0.078
Marital Status			
Married	575 (69.2)	2272 (68.4)	0.018
Not married	234 (28.2)	981 (29.5)	-0.030
Missing	22 (2.6)	71 (2.1)	0.033
Education			
Middle School or lower	163 (19.6)	711 (21.4)	- 0.044
High School	382 (46.0)	1471 (44.3)	0.034
University or upper	282 (33.9)	1118 (33.6)	0.006
Missing	4 (0.5)	24 (0.7)	-0.031
Employment			
Employed	665 (80.0)	2616 (78.7)	0.033
Not employed	165 (19.9)	708 (21.3)	-0.036
Missing	1 (0.1)	0 (0.0)	0.049
Type of conception			
Spontaneous	799 (96.1)	3180 (95.7)	0.024
Assisted	29 (3.5)	132 (4.0)	- 0.025
Missing	3 (0.4)	12 (0.4)	0.0
History of diabetes			
No	822 (98.9)	3307 (99.5)	- 0.067
Yes	9 (1.1)	17 (0.5)	0.067
History of hypertension			
No	800 (96.3)	3233 (97.3)	- 0.057
Yes	31 (3.7)	91 (2.7)	0.057

Source: [120]

Table 7 reports PR of selected adverse perinatal outcomes in women diagnosed with PAC. Diagnoses during pregnancy were associated with an increased risk of labor induction or planned delivery (PR=1.80, 95% CI: 1.57-2.07), cesarean section (PR= 1.78, 1.49–2.11), and preterm birth (aPR=6.34, 4.59-8.75). When only diagnoses during the year postpartum were considered, no association with obstetric outcomes emerged. For preterm deliveries, PAC women had a significantly higher proportion of induced deliveries or elective caesareans (70.3% vs 46.4%, p-value<0.001).

Table 7. Prevalence ratio (PR) of selected adverse perinatal outcomes in women diagnosed with pregnancy-associated cancer (PAC), with corresponding 95% confidence interval (95%CI). Lombardy, 2008-2017.

	Labour induction		Iatrogenic delivery		Preterm birth	
	PR	95%CI	PR	95%CI	PR	95%CI
Cancer-free	Ref.		Ref.		Ref.	
PAC during gestation	1.80	1.57-2.07	1.78	1.49-2.11	6.34	4.59-8.75
PAC postpartum	1.08	0.98-1.20	1.03	0.91-1.17	1.18	0.90-1.55

Modified from: [120]

No associations between PAC diagnosed during pregnancy (PR=0.71, 95%CI: 0.36-1.35) or in the year postpartum (PR=1.04, 95%CI: 0.78-1.39) and small for gestational age emerged. However, neonates had a lower mean birth weight in women with PAC compared to women without cancer (3109±588 g vs 3232±522 g, p-value< 0.0001). Furthermore, a lower mean birth weight was observed in PAC diagnosed during pregnancy compared to PAC diagnosed after pregnancy (2853±578 g vs 3183±570 g, p-value< 0.0001). Newborns of PAC women diagnosed during pregnancy had a higher risk, even if of borderline significance, of a low Apgar score (PR=2.65, 95%CI: 0.96-7.33) as compared to cancer-free women; however, no association was observed for women with PAC diagnosed in the year after birth (PR=0.66, 95%CI: 0.30-1.46). No cases of birth defects were selected in the group of PAC women, but 3 cases were observed in the cancer-free group. As regard perinatal mortality, a case in PAC women and 2 cases in cancer-free women emerged. In the PAC group, 27 (14.5%) women were exposed to chemotherapy. No association was found between adverse neonatal outcomes and having received chemotherapy. However, chemotherapy was found to influence birth weight. The mean birth weight is lower in infants exposed to chemotherapy compared to those not exposed (2462.33 ± 464.65 g and 2919.04 ± 570.62 g, respectively, p-value<0.001).

Some differences in selected outcomes emerged when stratified by the most common cancer site (i.e. breast, thyroid and lymphoma). For PAC diagnosed during pregnancy, elective caesarean section was more common in breast and lymphoma cases than in thyroid cases (respectively 82.1% vs. 82.4% vs. 52.9%, p value = 0.048), preterm delivery was more common in breast and lymphoma cases than in thyroid cases (respectively 52. 2% vs 44.4% vs 17.6%, p-value = 0.037), and small for gestational age was more common in thyroid cancer and lymphoma than in breast cancer (5.1%, 11.1% and 0%, respectively, p=0.036). Among PAC diagnosed after pregnancy, there were no differences between strata of cancer site.

4.1.1.3 Discussion and conclusion

If a tumour is diagnosed during pregnancy, the management of the birth is a critical consideration. The decision about the timing of delivery and whether to have a vaginal or caesarean section depends on a number of factors, including the type and stage of the tumour, its location, the general health of the mother and baby, and the potential risks involved. To ensure the best possible outcome for both mother and baby, this complex decision-making process requires close collaboration between oncologists, obstetricians, and other relevant medical specialists. In the field of oncology during pregnancy, a primary indication for caesarean section is abdominal-pelvic or cervical cancer [121]. However, whenever possible delivery management should be on the basis of obstetric indication, with efforts being made for term delivery [16]. In particular, in the context of a planned birth, each additional week of gestation towards the end of pregnancy reduces the risk of adverse neonatal outcomes.

In spite of this premise, elective caesarean section has been reported to be the preferred mode of delivery for women with cancer associated with pregnancy [4,122,123]. According to literature, the current investigation suggested that the diagnosis of PAC was positively related to planned labour and delivery, caesarean section, and preterm birth. The elevated rate of birth interventions observed in women diagnosed with PAC underscores the intricate nature of clinical decision-making, aiming to achieve a well-balanced outcome for both mothers and their infants [124]. For women with cancer, deciding for a planned cesarean section may offer enhanced control over timing both the cancer treatment and childbirth. This approach could mitigate the uncertainties linked to spontaneous labor and vaginal birth, thereby affording clinicians greater control over the process. Thus, an iatrogenic or induced preterm birth may have been chosen to allow treatment of the cancer to begin. However, it is also important to bear in mind that preterm birth, regardless of the concurrent oncological condition, may have an impact on the well-being of the newborn [4,122,123,125] and may therefore not be the optimal choice. In fact, women diagnosed with PAC are at increased risk of experiencing significant maternal morbidities due to their oncological condition, while their infants often encounter unfavorable perinatal outcomes linked to preterm birth [124]. In the present study, an higher risk for low birth weight and low Apgar score emerged in women with a diagnosis of PAC compared to those without, probably driven by the increased frequency of preterm and iatrogenic birth. In this direction, there is increasing awareness that cancer is treatable during pregnancy and the proportion of patients receiving treatment during pregnancy is increasing [21,31]. Antenatal treatment of cancer should limit iatrogenic prematurity [16,126].

In the proposed study, only a small proportion of women with pregnancy-related cancer were treated with chemotherapy, and chemotherapeutic treatment has been suggested to have an negative effect on birth weight. The administration of chemotherapy during pregnancy requires careful consideration of trimester timing. Ideally, chemotherapy should be avoided in the first trimester because of the increased risk of fetal malformations. As pregnancy progresses into the second and third trimesters, the association between chemotherapy and birth defects decreases [127,128]. Nevertheless, it is crucial to note that in these later stages, chemotherapy could increase the risk of intrauterine growth restriction, preterm birth, low birth weight, and even stillbirth [127]. Particularly noteworthy is the association between prenatal exposure to chemotherapy and an increased susceptibility to intrauterine growth restriction [129]. With this in mind, it is imperative that pregnant women diagnosed with cancer receive specialised care in a high-risk obstetric unit. This care should emphasise close surveillance monitoring of fetal growth, a critical focus that becomes even more important in the third trimester.

In the study in question, the diagnosis of PAC was associated with iatrogenic preterm birth. As a result, the newborns of mothers with cancer had a lower birth weight and a worse Apgar score compared to those without cancer. This reflects the fact that the adverse neonatal outcomes were, at least in part, due to the prematurity of the baby. To ensure the well-being of neonates born to women with PAC, particular attention

must be paid to the timing of delivery. The imperative is to minimise iatrogenic preterm birth, a measure that should be undertaken with caution, given the delicate balance between maternal and fetal health.

4.1.2 II Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Franchi M, Santucci C, Scarfone G, Parazzini F, La Vecchia C, Corrao G, Negri E. Spontaneous and induced abortions in women with a diagnosis of gestational related neoplasm: a population-based linkage study in Lombardy, 2010-2020. BMC Womens Health. 2023 Nov 8;23(1):586. doi: 10.1186/s12905-023-02685-6 [130]**

4.1.2.1 Specific aim and methods

Differently from the study described above, the following research addresses a broader question. This particular study has widened to include all pregnancies, regardless of their outcome, whether birth or abortion. This shift in perspective stems from the core aim of the study: to examine the complex relationship between cancer and abortion, and to provide a comprehensive assessment of this multifaceted relationship.
The general focus of this study was to examine in detail the temporal trends in cancer incidence, particularly in relation to pregnancy outcomes. The study looked at how the incidence of cancer has changed over time in women who had abortions and women who gave birth. The specific aim was to assess the association between the diagnosis of PAC and abortion.

For this study, in a cohort of women hospitalised for childbirth between 2010 and 2020, mothers were identified as having PAC if they had at least one hospitalisation with main or secondary diagnosis of cancer during gestation up to one year after delivery or abortion. Only incident cases were considered, excluding women with a diagnosis of cancer in the three years before conception. According to the World Health Organisation (WHO), abortion is defined as the termination of pregnancy before 22 weeks' gestation. All spontaneous (ICD-9-CM codes: 632. and 634.) or induced abortions (ICD-9-CM code: 635.) among hospitalised women were identified, both those without dilation and curettage (DRG: 380) and those with dilation and curettage, aspiration or hysterectomy (DRG: 381). Fetal death later in pregnancy was considered a delivery with perinatal death.

The incidence rate of PAC per 1000 pregnancies was calculated by dividing the observed number of cases by the total number of identified pregnancies. The incidence rate was calculated also separately for abortions and deliveries. The Cochran-Armitage test for trend was used to assess a linear trend in the incidence rate of PAC over the period observed. The Chi-squared test was used to test for differences in proportion of deliveries and abortions between women with diagnosis of cancer and those without.

Log-binomial regression models were fitted to estimate the PR and the corresponding 95%CI of abortions associated with maternal diagnosis of PAC, including term of maternal age.
Timing of diagnosis was taken into account and cases were stratified into those diagnosed in the first trimester and those diagnosed after.

4.1.2.2 Results

In the cohort of women who gave births, 926 (1.29 per 1000 births) were diagnosed with PAC, about 20% during pregnancy and 80% in the postpartum. While, in the cohort of women who aborted, 341 (1.35 per 1000 births) were diagnosed with PAC, about 10% during gestation and 90% in the postpartum, especially in the first months. **Figure 1** shows the trend in the incidence rate per 1000 in the two groups. No trend was observed overall; however, a decreasing in diagnoses of PAC emerged for women who aborted in the period 2019-2020. The most common cancer sites were breast, thyroid, lymphomas, and cervix cancer.

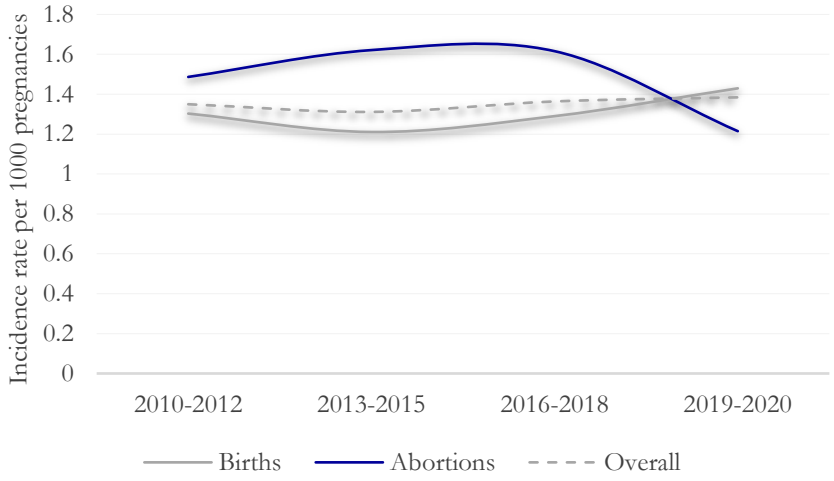

Figure 1. Temporal trends of incidence rate (per 1000 pregnancies) of pregnancy-associated cancers (PAC) in the cohort of births and in the cohort of abortions. Lombardy, 2010-2020.

Figure 2 describes the distribution of births and spontaneous and induced abortions in women diagnosed with pregnacy-associated cancer by calendar period. The diagnosis of PAC was associated with an increased risk of miscarriage of about 10% (PR=1.11, 95%CI: 1.01-1.22). While considering only diagnoses performed in the first trimester, the risk was higher (PR=2.53, 95%CI: 2.05-3.11), in particular for induced abortions (PR=3.71, 95%CI: 2.82-4.90); considering only those performed after the first trimester no association emerged (PR=1.04, 95%CI: 0.95-1.15).

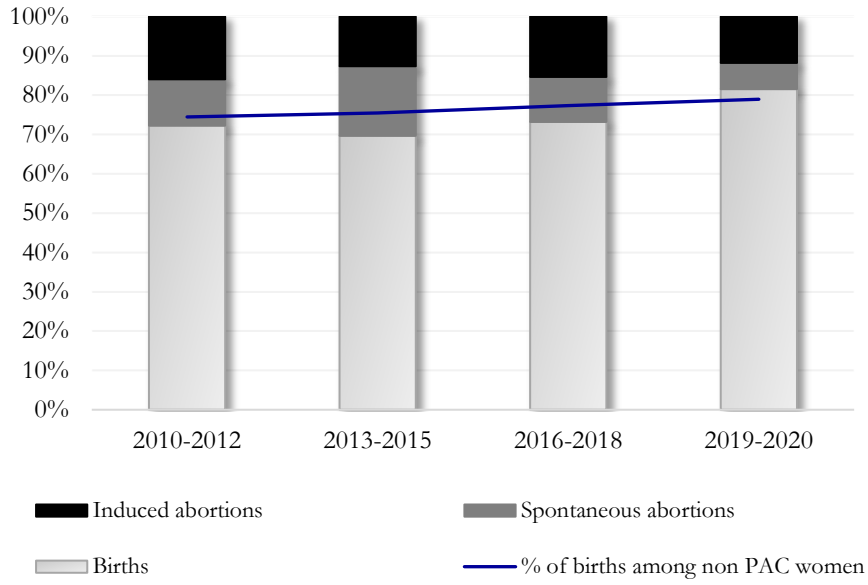

Figure 2. Pregnancy outcomes in the cohort of women diagnosed with pregnancy-associated cancers (PAC). Lombardy, 2010-2020.

Table 8 reports PR of abortion in women diagnosed with PAC according to selected calendar periods. In the first period, especially between 2013 and 2015, the risk of abortion was increased in the group of women with PAC compared to women without (PR=1.21, 95%CI: 1.03-1.42). The association disappeared in the last two years considered (PR=0.87, 95%CI: 0.65-1.17), but was higher and remained significant throughout the study period when only PACs diagnosed in the first trimester of pregnancy were considered.

Table 8. Prevalence ratio (PR) and corresponding 95% confidence intervals (CI) of abortion in women diagnosed with pregnancy-associated cancer (PAC). Lombardy, 2010-2020.

		PAC women, PR[a] (95%CI)		
	Cancer-free women	All the diagnoses	Diagnoses during the first trimester	Diagnoses during the second trimester or later
2010-2012	Ref.	1.08 (0.92-1.26)	2.77 (2.08-3.69)	0.99 (0.84-1.18)
2013-2015	Ref.	1.21 (1.03-1.42)	1.99 (1.18-3.36)	1.18 (1.00-1.39)
2016-2018	Ref.	1.17 (0.98-1.40)	2.68 (1.83-3.91)	1.09 (0.90-1.32)
2019-2020	Ref.	0.87 (0.65-1.17)	2.67 (1.41-5.07)	0.80 (0.59-1.10)

[a] Estimated from log-binomial regression model adjusted for maternal age.

4.1.2.3 Discussion and conclusion

The study found that a diagnosis of PAC was associated with an increased risk of miscarriage of about 10%. However, it is worth noting that this association seemed to

be decreasing in more recent calendar years. This trend seemed to be driven by spontaneous abortions rather than induced abortions.

The link between abortion and PAC has not been extensively explored, and the findings in this regard have not shown consistent results. An investigation including Italian women from various regions (Veneto, Piedmont, Tuscany, and Apulia) found that the risk of PAC was slightly lower in women who aborted compared to women who delivered (1.25 vs 1.35 per 1000) [131]. However, another study from Lombardy region reported that the risk of being diagnosed with PAC was about 20% higher in pregnancies that ended in a termination of pregnancy compared with those that ended in a birth [2]. In line with this, in Apulia, about 60% of pregnancies of women affected by cacner ended with an abortion [3].

In the context of a cancer diagnosis, the issue of abortion should be considered in terms of both clinical implications and personal choices. Since organogenesis occurs in the first trimester, the use of chemotherapy in this early phase of pregnancy is associated with an increased risk of miscarriage, fetal death and congenital malformations [22]. However, the association between chemotherapy and miscarriage and birth defects decreases during the second and third trimesters [127,128]. Furthermore, the approach to cancer in pregnancy has improved over time, with better management of clinical cases [132-134]. As a result, cancer treatment has been favoured instead of termination of pregnancy, and an higher number of live births from women with cancer has been reported over time [132,135]. There has also been an increase in the number of pregnant women treated with chemotherapy, highlighting an increased awareness of the feasibility and safety of oncological treatment during pregnancy [136].

As for the second aspect regarding the personal decision to interrupt pregnancy by woman or couple. With regard to the second aspect, which concerns the individual decision of the woman or the couple to terminate a pregnancy, it is important to note that it is difficult to argue this point comprehensively when discussing cancer diagnoses because of the unavailability of information on the voluntary nature of the termination, the motivations behind it, and the clinical conditions.

Over the recents years, thanks to advances in the field of diagnostic methods and clinical management tools, a pregnant cancer patient may not compromise her chances of survival without giving up on pregnancy. The availability and timing of treatment options is influenced by the location of the tumour, its spread and aggressiveness, the gestational age at diagnosis, and the patient's choice.

4.2 Assisted reproductive technologies

4.2.1 III Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Somigliana E, Franchi M, Dallagiovanna C, Pisaturo V, Corrao G, Parazzini F. Trend of medically induced monozygotic twin deliveries according to age, parity, and type of assisted reproductive technique during the period 2007-2017 in Lombardy Region, Northern Italy: a population-based study. J Assist Reprod Genet. 2021 Sep;38(9):2341-2347. doi: 10.1007/s10815-021-02268-0 [137]**

4.2.1.1 Specific aim and methods

Although the determinants remain poorly understood, the risk of monozygotic twinning is increased in pregnancies following ART [138]. The problem of monozygotic pregnancies, which had been hidden for years, has been revealed by the progressive diffusion of single embryo transfer and the consequent reduction in the incidence of dizygotic pregnancies. The prevention of monozygotic pregnancies in the context of ART is the new challenge and is the reason for the joint efforts of the scientific community.
The study in exam provided frequency and temporal trends over time ART-mediated monozygotic twinning, with the additional aim of assessing the association of monozygotic twinning with maternal age, parity, and different types of ART.

Only ART-mediated births occurred between 2007 and 2017 were included in this analysis, excluding triplets and quadruplets.

Once the total number of sex-concordant and sex-discordant twin births had been determined, Weinberg's method was used to estimate the dizygotic and monozygotic birth rates [139]. In summary, it has been assumed that sex is distributed independently in dizygotic pregnancies compared to monozygotic pregnancies. Thus, an estimate of the number of monozygotic twins is given by the difference between the total number of twins and twice the number of discordant twins. The estimated number of monozygotic and dizygotic twins was divided by the total number of deliveries to calculate the overall rate of monozygotic and dizygotic twin births.

Standardised rates (SR) for age were calculated using a direct method of standardisation across strata of parity and type of ART, i.e. first level techniques, conventional in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI).

Chi-squared was used for testing differences in SR. Temporal trends were examined by dividing the study period into three intervals (2007-2010, 2011-2014 and 2015-2017) and using Chi-squared test for trend.

4.2.1.2 Results

A total of 19,130 ART-mediated births were included, 15,684 (82.0%) singleton and 3446 (18.0%) twin births. Over the ten-year study period, the frequency of twin births was 18.0% and 1.2% for ART and natural conception, respectively.
Based on Weinberg's rule, the overall estimated rate of ART-mediated monozygotic births was 1.02 per 100 births (95%CI: 0.89-1.17) and showed a decreasing trend (p-value=0.03), with age-adjusted SR of 1.33 (95%CI: 1.18-1.51), 0.96 (95%CI: 0.83-1.11) and 0.92 (95%CI: 0.79-1.07) in the periods 2007-2010, 2011-2014, and 2015-2017, respectively. **Figure 3** shows the time trend of ART-mediated monozygotic births, observed for three-year intervals.

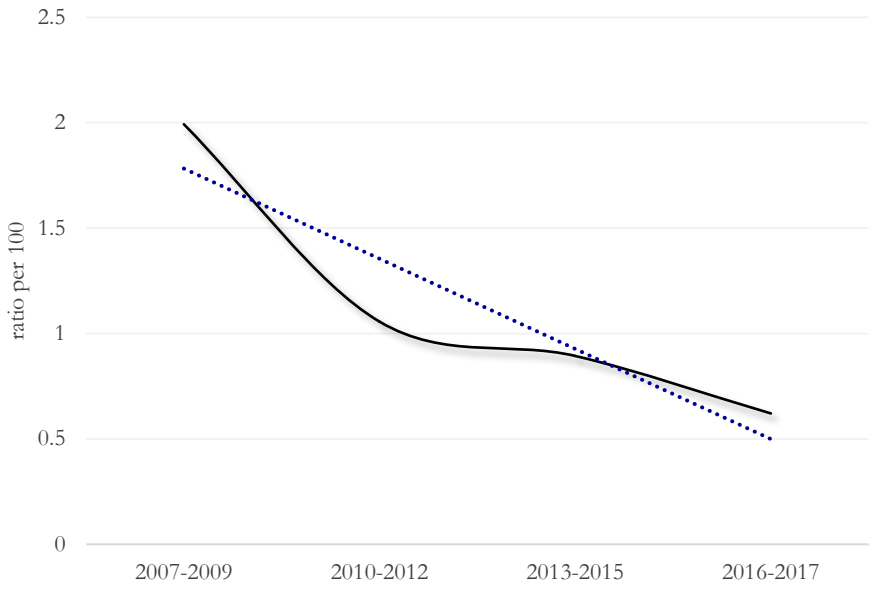

Figure 3. Temporal trend of monozygotic twin births after assisted reproductive technologies (ART). Lombardy, 2007-2017. (Modified from: [137])

The rate was significantly higher among women younger than 35 years (1.51, 95%CI: 1.24-1.84) when compared with older ones (0.78, 95%CI: 0.64-0.94) (**Table 9**). This association was consistent over the three periods, but not significant in the period 2007-2010 (p-value=0.09).
Table 10 shows the frequency of monozygotic twins by parity. There were 7855 (41.1%) missing values for parity. The SR adjusted for maternal age were 0.72 (95%CI: 0.61-0.85) in multiparous women and 0.97 (95%CI: 0.84-1.12) in nulliparous ones.

Table 9. Rate per 100 and standardized rate (SR) of monozygotic twin births according to calendar period and maternal age. Lombardy, 2007-2017.

Calendar period	N	Rate per 100	SR (95%CI)	p-value
2007-2010				
< 35 years	28	1.72 (1.19-2.47)	1.33 (1.18-1.51)	
≥ 35 years	28	1.13 (0.78-1.62)		
2011-2014				
< 35 years	41	1.55 (1.15-2.10)	0.96 (0.83-1.11)	0.03
≥ 35 years	34	0.66 (0.47-0.92)		
2015-2017				
< 35 years	29	1.31 (0.91-1.87)	0.92 (0.79-1.07)	
≥ 35 years	36	0.72 (0.52-0.99)		

Source: [137]

Table 10. Rate per 100 and standardized rate (SR) of monozygotic twin births according to parity and maternal age. Lombardy, 2007-2017.

Parity	N	Rate per 100	SR (95%CI)	p-value
Parous women				
< 35 years	13	1.58 (0.93-2.69)	0.72 (0.61-0.85)	
≥ 35 years	6	0.27 (0.13-0.60)		
Nulliparous women				<0.01
< 35 years	47	1.61 (1.21-2.13)	0.97 (0.84-1.12)	
≥ 35 years	34	0.64 (0.46-0.89)		

Source: [137]

Table 11. Rate per 100 and standardized rate (SR) of monozygotic twin births according to method of assisted reproductive technologies (ART) and maternal age. Lombardy, 2007-2017.

Method of ART	N	Rate per 100	SR (95%CI)	p-value
First level techniques				
< 35	7	0.47 (0.23-0.96)	0.47 (0.38-0.57)	
≥ 35	7	0.47 (0.23-0.96)		
IVF				
< 35	38	1.98 (1.45-2.71)	1.02 (0.88-1.17)	<0.01
≥ 35	23	0.52 (0.34-0.78)		
ICSI				
< 35	48	1.89 (1.43-2.50)	1.43 (1.27-1.61)	
≥ 35	62	1.19 (0.93-1.52)		

Source: [137]

The frequency of monozygotic twin according to type of ART is shown in **Table 11**. The SR adjusted for maternal age were 0.47 (95%CI: 0.38-0.57) among women who underwent first-level techniques. This was similar to that observed for natural

conception. On the other hand, higher SR were observed in women who underwent IVF and ICSI (1.02, 95%CI: 0.88-1.17 and 1.43, 95%CI: 1.27-1.61, respectively). In both the IVF and ICSI groups, the monozygotic twin rate was higher in women younger than 35 years compared to older women. In the group of women who underwent first-stage techniques, this was not observed.

4.2.1.3 Discussion and conclusion

The study showed that the risk of monozygotic twin births was two-fold increased in women who underwent ART compared to those conceived naturally, with a decreasing trend over time. According to regional data, monozygotic twinning rate was 1.02 per 100 births in women undergoing ART compared to about 0.40 in those who conceived spontaneously. The risk was higher in women who underwent IVF or ICSI and in younger women.

The downward trends are novel and intriguing, even if the explanations are difficult. In the last 10-15 years, the most significant change in the field of ART has been the use of frozen transfer. In Lombardy, the annual number of frozen embryo transfer cycles increased progressively from 102 in 2007 to 5455 in 2017 [47]. This practise, regardless developmental stage, has been associated to a lower monozygotic twinning [140]. The role of the endometrial environment in fresh embryo transfer may explain this association. During the implantation phase, embryos transferred in fresh cycles are exposed to a non-physiological environment, which may cause some disturbances and ultimately facilitate monozygotic twinning. The clinical implications of this, if confirmed, could be an additional argument in favour of a frozen transfer strategy.

Other plausible explanations include changes in laboratory conditions that may have occurred gradually over the study period in the ART clinics in Lombardy, such as changes in incubators, medical staff expertise and transfer strategies. Micromanipulation of the zona pellucida [141,142], performed in ICSI or assisted hatching, and blastocyst transfer [140] may impact on the monozygotic twinning. In line with this, the present investigation found that women receiving ICSI were at the highest risk of monozygotic twinning. Over the study period, the use of assisted hatching has decreased, in line with growing awareness that this intervention is ineffective in improving pregnancy rates [143]. In addition, according to data analysed, an increase in the use of IVF rather than ICSI procedures was observed. However, there is likely to have been a concomitant increase in the transfer of blastocysts rather than cleavage stage embryos.

The current study confirmed a two-fold monozygotic twinning risk in young maternal age (<35 versus ≥35 years). This finding is consistent with literature [138,144]. The association was not observed in patients who achieved natural conception and those who underwent first stage techniques, suggesting that young age is not a direct determinant of monozygotic pregnancy. The link between young age and monozygotic twinning risk is still poorly understood. The availability of better quality embryos may

explain the increase in monozygotic pregnancies in younger women [145]. Others have suggested that twin embryos from older mothers may be more prone to chromosomal abnormalities and miscarriage before reaching clinical pregnancy [146].
To clarify the real determinants of monozygotic risk in ART pregnancies, future large and multicentre studies are urgently needed.
Some limitations of the present study should be acknowledged: the information on zygosity was not directly available but was inferred using a probabilistic method, the information recorded on the ART procedure performed was often imprecise (no details on the day of embryo transfer, assisted hatching or embryo biopsy performed are available in the administrative databases), women who resorted to oocyte donation were not identified.

4.2.2 IV Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Cipriani S, Noli S, Franchi M, Corrao G, Parazzini F, Somigliana E. The changing impact of assisted reproductive techniques on preterm birth during the period 2007-2020 in Lombardy, Northern Italy. Eur J Obstet Gynecol Reprod Biol. 2022 Nov; 278:51-56. doi: 10.1016/j.ejogrb.2022.09.003 [147]**

4.2.2.1 Specific aim and methods

Preterm birth, defined by the WHO as a birth before 37 completed weeks of gestation, is the leading cause of infant morbidity and mortality in wealthy countries such as Italy. ART has been associated to a higher risk of a preterm birth [148]. The high rate of twin pregnancies after ART may be an important explanation for this association. However, preterm birth is also associated with singleton pregnancies after ART.
The present study aimed at assessing the impact of ART on the risk of preterm birth, moving reflections on the effect of changing techniques.

All births occurred between 2007 and 2020 were included in this analysis.

Exposure. ART included first level techniques, such as pharmacological ovulation induction and IUI, ICSI, and conventional IVF.
Outcome. Preterm birth was defined as any birth after 22 weeks of gestation and before 37 weeks [149]. Preterm birth could be categorised according to gestational week, i.e. extremely preterm (<28 weeks), very preterm (28-31 weeks), moderate to late preterm (32-36 weeks) [150]. In the analysis, the first two categories were combined into one, as births before 28 weeks represent less than 5% of all births before 37 weeks.

Standard descriptive statistics, including frequencies and percentages for categorical variables and means and standard deviations for continuous variables, were calculated to assess the distribution of maternal sociodemographic and clinical characteristics according to exposure to ART. Chi-squared test was used to test for differences in categorical variables between the group of women who conceived after ART and the group of women who conceived naturally. Independent samples t-tests were used to compare numerical variables between the two groups.
Log-binomial regression models were fitted to estimate the PR and 95% CI of preterm birth associated with ART. Estimates were adjusted for socio-demographic characteristics, such as age, nationality, marital status, education, and employment. As sensitivity analysis, multiple births were excluded.
Finally, the population attributable fraction (PAF) - expressed in percentage - of ART to preterm birth was computed using the standard formula: [(risk of preterm birth in

all women - risk of preterm birth in women conceiving naturally) / risk of preterm birth in all women].
To evaluate temporal trends, study period was divided in biennia for all analyses. Simple linear regression was used to analyse trends in the proportion of ART across calendar years as a continuous variable.

4.2.2.2 Results

The cohort included 1,085,159 naturally conceived births and 28,742 ART-mediated births (25.8 per 1000 births). Over the calendar years, there was an important upward trend: the number of births after ART increased almost fourfold between 2007 (10.9 per 1000) and 2020 (38.7 per 1000) (p-value<0.001). As regard ART techniques, 39.1% were ICSI, 36.7% IVF, 13.8% IUI or pharmacological ovulation induction, and 10.4% not defined. Women who had a naturally conceived birth had a lower mean age than those who had an ART assisted birth (31.9±5.3 vs 36.3±4.5, p-value<0.001). Mothers who underwent ART were more likely to be educated and more likely to be of Italian nationality.
A total of 12,800 (1.2%) multiple births were identified in naturally conceived births and 4632 (16.1 %) in ART-mediated births. Over the study period, there was a decreasing trend in the number of multiple births after ART: the proportion of multiple births was 20.4% in 2007 and 8.4% in 2020.

Table 12. Prevalence ratio (PR) and corresponding 95% confidence intervals (CI) of preterm birth in ART-mediated births, overall and in the subgroups of singletons and multiples. Lombardy, 2007-2020.

Timing of delivery	ART, N (%)	Spontaneous, N (%)	PR (95%CI)	aPR (95%)
Overall births				
At term	23,558 (82.0)	1,018,392 (93.8)	Ref.	Ref.
Preterm	5184 (18.0)	66,767 (6.2)	2.93 (2.86-3.01)	2.83 (2.76-2.91)
< 32 w	829 (2.9)	8860 (0.8)	3.94 (3.67-4.23)	4.08 (3.79-4.39)
32-36 w	4355 (15.1)	57,907 (5.4)	2.90 (2.82-2.98)	2.76 (2.69-2.86)
Multiple births				
At term	1808 (39.0)	5230 (40.9)	Ref.	Ref.
Preterm	2824 (61.0)	7570 (59.1)	1.03 (1.00-1.06)	1.04 (1.01-1.07)
< 32 w	424 (9.2)	983 (7.7)	1.20 (1.08-1.33)	1.32 (1.18-1.48)
32-36 w	2400 (51.8)	6587 (51.4)	1.02 (0.99-1.06)	1.02 (0.99-1.06)
Singleton births				
At term	21,750 (90.2)	1,013,162 (94.5)	Ref.	Ref.
Preterm	2360 (9.8)	59,197 (5.5)	1.77 (1.71-1.84)	1.72 (1.65-1.79)
< 32 w	405 (1.7)	7877 (0.7)	2.37 (2.15-2.62)	2.45 (2.21-2.72)
32-36 w	1955 (8.1)	51,320 (4.8)	1.71 (1.64-1.79)	1.65 (1.57-1.72)

PR: prevalence ratio. aPR: adjusted prevalence ratio, estimated from log-binomial regression model adjusted for maternal sociodemographic features (age, nationality, marital status, education and employment). (Source: [147])

Table 12 shows the PR of preterm birth for ART for all births and in the subgroups of singletons and multiples. ART, regardless type of technique, was positively associated with preterm birth (PR=2.83, 95%CI: 2.76-2.91). In particular, a higher association between ART and births less than 32 weeks rather than births between 32 and 36 weeks emerged (PR=4.08, 95%CI: 3.79-4.39 and PR=2.76, 95%CI: 2.69-2.86, respectively). The mean gestational age was 33.79 weeks for ART-mediated preterm births and 34.17 weeks for natural preterm births (p-value<0.001). When multiple births were excluded, ART was still associated with preterm birth, but the association decreased significantly (PR=1.72, 95 % CI: 1.65-1.79). Instead, when only multiple births were considered, ART was associated only with preterm births before 32 weeks (PR=1.32, 95 %CI: 1.18-1.48).

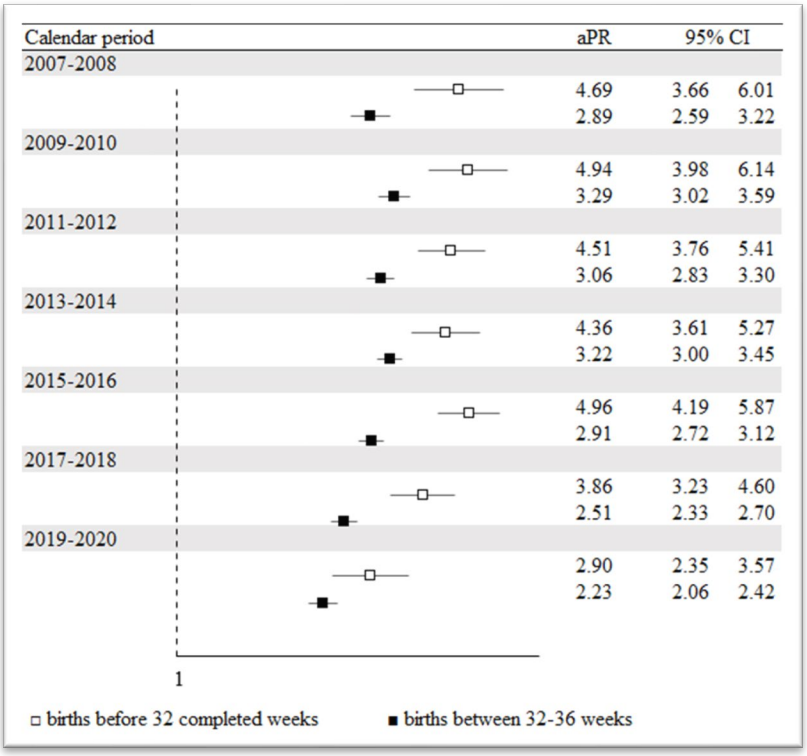

Figure 4. Prevalence ratios (PR) and corresponding 95% confidence interval (CI) of preterm birth for birth after assisted reproductive techniques (ART) according to timing of delivery (weeks) and calendar period of birth. Lombardy, 2007-2020. (Source: [147])

Figure 4 shows the trend of the association between ART and preterm birth by biennium from 2007 to 2020. The association was greater in the first period of the study, a decreasing trend emerged from 2017: the highest PR (95%CI) was observed in 2015-2016 with 4.96 (4.19-5.87) and the lowest was 2.90 (2.35-3.57) in 2019-2020.
As expected, there was a significantly higher proportion of preterm births in multiples than in singletons (59.1% vs 4.1% for naturally conceived births and 61.0% vs 9.8% for ART-mediated births). When multiple births were excluded, the association

between ART and preterm birth was significantly lower and did not describe a trend. **Figure 5** shows the trend of the association between ART and preterm birth by biennium from 2007 to 2020 in singleton births. The risk of birth before 32 completed weeks was 2-3 times higher in ART-mediated births, and the risk of birth between 32 and 36 weeks was 1.5 times higher.

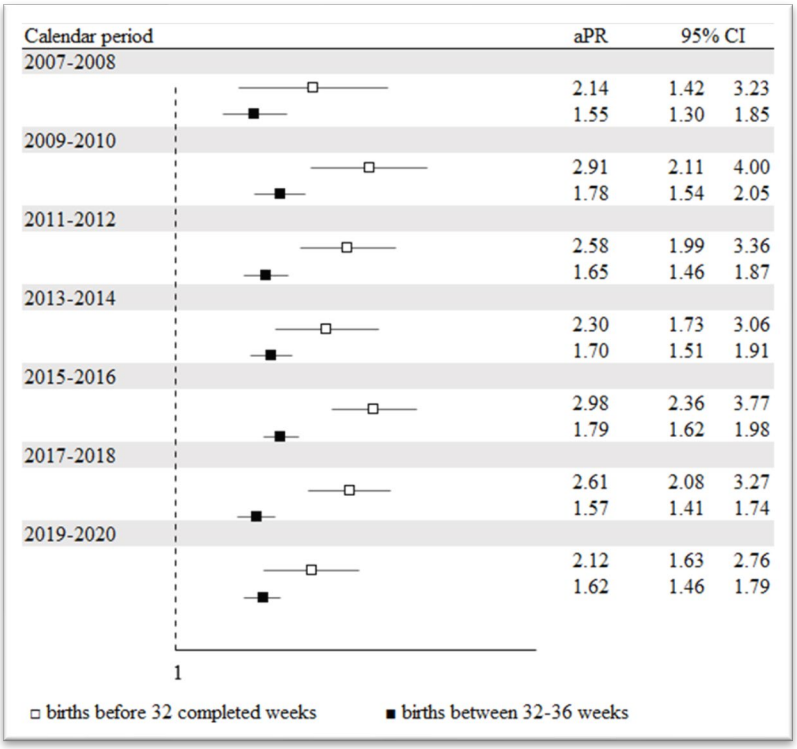

Figure 5. Prevalence ratios (PR) and corresponding 95% confidence interval (CI) of preterm birth for birth after assisted reproductive techniques (ART) among singleton births according to timing of delivery (weeks) and calendar period of birth. Lombardy, 2007-2020. (Source: [147])

Table 13 shows the PAF% of the preterm birth in the different calendar periods considered. The contribution of ART to preterm birth increased from 2007-2008 to 2015-2016 and then decreased.

Table 13. Population attributable fraction (PAF) of assisted reproductive technologies (ART) to preterm birth. Lombardy, 2007-2020.

	PAF (%)		
Calendar period	All preterm	<32 weeks	32-36 weeks
2007-2008	2.3	3.4	2.2
2009-2010	3.6	4.9	3.5
2011-2012	4.7	6.7	4.5
2013-2014	5.9	6.5	5.9
2015-2016	6.4	9.2	6.1
2017-2018	5.9	8.7	5.5
2019-2020	4.9	6.2	4.7

Source: [147]

4.2.2.3 Discussion and conclusion

There was a clear upward trend in the frequency of ART-mediated births over the study period, with an almost fourfold increase. Women who underwent ART procedures had an increased risk of PTB, even after excluding multiple pregnancies, which have a known increased risk associated with PTB.

More than 50% of the twin births terminated before the end of pregnancy [151,152], regardless the type of conception [153]. Evidence regarding perinatal outcomes of ART-mediated twins shows conflicting results. While some authors have shown similar gestational age and perinatal outcomes in natural and ART-mediated twins [154,155], others have reported a slightly higher risk of preterm birth in ART-mediated ones [156,157]. In addition, other studies have shown a higher prevalence of obstetric and perinatal complications (e.g. unplanned hysterectomy, postpartum haemorrhage and maternal blood transfusion, intensive care admission, preterm premature rupture of membranes, placenta accreta spectrum) in twins after ART compared with naturally conceived ones [158,159]. Therefore, as an effective strategy to reduce the risk of preterm birth, among other complications, recent evidence highlights the importance of reducing multiple pregnancies in assisted reproduction [70,160]. In the analysed cohort, the proportion of multiple births after ART declined from approximately 20% in 2007 to 8% in 2020.A recent report from several parts of the world for the period 1997-2016 showed a decline in the frequency of multiple births following IVF in each of the countries studied, reaching a minimum rate of around 4% in Australia and New Zealand [69]. New procedures (such as the increasing use of frozen embryo transfer and cryo cycles and the gradual introduction of freeze-all) and the resulting reduction in the number of embryos replaced per treatment attempt may partly explain this trend. A meta-analysis showed that the odds of having a multiple live birth were significantly lower in women who were randomly assigned to receive an elective single embryo transfer than in women who received a double embryo transfer [72]. Indeed, other ART procedures (e.g., extended embryo culture and late stage transfer) may be associated with this outcome, and spontaneous zygote splitting after embryo transfer but before uterine implantation may occur [73].

In the present study, a notable occurrence of preterm births was observed among women who conceived through ART. However, from 2007 to 2020, there was a gradual decline in the association between ART and preterm births. This can be attributed to the decreasing rate of multiple births following ART during this period. The confirmation of a consistently lower preterm births risk in singletons further supports this hypothesis. However, even the singleton pregnancies that resulted from these technologies are at a higher risk of complications [161,162], including preterm birth [163]. Several hypotheses have been made regarding the underlying mechanisms; in particular, abnormal placentation and possible consequences, such as fetal hypoxaemia or chronic placental inflammation, may contribute to the pathogenesis of spontaneous preterm birth in ART-mediated pregnancies [163]. The high levels of estradiol produced by ovulation induction have been suggested to be detrimental to

implantation, placentation, and fetal growth [164,165]. Furthermore, subfertile women conceiving without ART have been reported to have increased risk of preterm birth and other adverse outcomes [166,167].

Finally, as regards the study of the impact of ART on preterm birth through the PAF%. At first, there was an upward trend, which could be explained by the continuous increase in the frequency of ART, largely but not entirely compensated by the decrease in multiple births. In the period 2017 to 2020, a decrease in the PAF% was observed, mainly because of the stronger decrease in the frequency of multiples and a sudden reversal of the increasing trend of ART use in this last period, presumably because of the Covid-19 pandemic [168].

In conclusion, the association between ART and preterm birth is mainly due to the high proportion of multiple births among women who conceived after ART. The burden of preterm birth and related complications could be effectively reduced by limiting the rate of multiple births. Most importantly, the population-attributable risk of ART remained largely stable over the study period, despite a significant increase in the number of ART babies, as stakeholders became more aware of the obstetric complications of multiple pregnancies and treatment strategies changed accordingly. In singleton pregnancies, however, ART was also associated with preterm birth. Therefore, there is still room for significant improvement and further studies are needed in order to better investigate the determinants of preterm birth and innovative treatment strategies.

4.2.3 V Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Cantarutti A, Mauri PA, Franchi M, Fedele F, Corrao G, Parazzini F, Persico N. Prevalence and Factors Associated With Intertwin Birth Weight Discordance Among Same-Sex Twins in Lombardy, Northern Italy. Twin Res Hum Genet. 2023 Apr;26(2):177-183. doi: 10.1017/thg.2023.17 [169]**

4.2.3.1 Specific aim and methods

A twin pregnancy is considered a health concern, as it is widely recognized to carry a higher risk of maternal and fetal complications, as well as perinatal mortality, compared to a singleton pregnancy. Over the past four decades, the prevalence of twinning, especially dizygotic twinning, increased significantly in most developed countries as ART became more widely available. Birth weight discordance (BWD), defined as a 15% to 40% difference in birth weight between the larger and smaller twin, is an adverse condition typical of twin pregnancies.
The purpose of the study described below was to investigate the prevalence, potential risk factors, and consequences of BWD in same-sex twins.

For this study, only same-sex twins born between 2007 and 2021 were considered.

Intertwin discordance was obtained by the formula:

$$100 * \frac{larger\ twin\ weight - smaller\ twin\ weight}{larger\ twin\ weight}$$

According to previous evidence [170,171], pregnancy was considered complicated by BWD if the disparity was 30% or more.

Univariate and multivariate logistic regression models were fitted to determine the potential factors (i.e., maternal age, educational level, nationality, modality of conception, and parity) associated with BWD, estimating the OR and 95% CI. Analysis was repeated in strata by sex.
The adverse neonatal outcomes considered were birth weight of 2500 grams or less, preterm birth, small for gestational age (according to sex-specific Italian reference curve for normal fetal growth [172]), Apgar score of 7 or less, perinatal mortality. We compared the distribution of these outcomes according to the BWD level (i.e. less than 20%, between 21% and 29%, equal to 30% or more), testing the differences using the trend test. Analysis was repeated excluding 7009 preterm births. Finally, RR and 95% CI, adjusted for maternal age, educational level, nationality, and parity, of the adverse neonatal outcomes and the mode of conception according to BWD was computed.

4.2.3.2 Results

A total of 11,096 same-sex twin births were included in the analysis, 5620 (50.6%) couples of female twins and 5476 (49.4%) couples of male twins.

Males were heavier than females, with a mean birth weight of 2309.2 g and 2232.8 g respectively (p-value<0.001). The number of births affected by BWD was 556 (5.0%), 279 (5.0%) for females and 277 (5.0%) for males (p-value=0.820). Specifically, 6175 (55.7%) twin deliveries had discordance of less than 10 %, 3144 (28.3%) between 10 and 19 %, 1221 (11.0%) between 20 and 29 %, 377 (3.4%) between 30 and 39 % and 179 (1.6%) over 40 %.

Table 14. Odd ratio (OR) and corresponding 95% confidence intervals (CI) of birthweight discordance (BWD) cases according to selected potential risk factors. Lombardy, 2007-2021.

	BWD	Univariate model	Multivariate model
	n (%)	OR (95%CI)	OR (95%CI)
Maternal age			
<35	289 (4.5)	Ref.	Ref.
>35	267 (5.6)	1.26 (1.06-1.49)	1.26 (1.05-1.51)
Nationality			
Italian	434 (5.1)	Ref.	Ref.
Foreign	122(4.7)	0.92 (0.75-1.13)	0.99 (0.79-1.22)
Level of education[a]			
University	181 (4.7)	Ref.	Ref.
High school	237 (5.0)	1.07 (0.87-1.30)	1.12 (0.92-1.37)
Middle/primary school	137 (5.5)	1.18 (0.94-1.48)	1.34 (1.05-1.70)
Parity			
Nulliparous	377 (5.6)	Ref.	Ref.
Parous	179 (4.1)	0.73 (0.61-0.88)	0.73 (0.60-0.89)
Conception			
Spontaneous	402 (4.7)	Ref.	Ref.
Non-spontaneous	154 (6.1)	1.33 (1.10-1.61)	1.16 (0.94-1.44)

[a] 9 missing values (Source: [169])

Table 14 shows OR of BWD cases according to selected potential risk factors. Maternal age over 35 years (OR=1.23, 95%CI: 1.01-1.50), parous status (OR=0.73, 95%CI: 0.61-0.88) and non-spontaneous conception (OR=1.33, 95%CI: 1.10-1.61) were significant factors for BWD in univariate analysis. After adjustment for all potential risk factors, gestational age over 35 years (OR=1.26, 95%CI: 1.05-1.51), low educational level (OR=1.34, 95%CI: 1.05-1.70) and parous status (OR=0.73, 95%CI: 0.60-0.89) were significantly associated with BWD. When the discordance cut-off was lowered to 20%, in the multivariate analysis, advanced maternal age (OR=1.19, 95%CI: 1.07-1.33) and ART (OR=1.17, 95%CI: 1.03-1.32) remained associated with an increased risk of BWD, and parity (OR=0.77, 95%CI: 0.69-0.87) with a decreased risk.

Table 15. Distribution of mode of delivery and adverse neonatal outcomes by degree of weight discordance. Lombardy, 2007-2021.

	BWD			p-value for trend
	<20%	20-29%	≥30%	
	(N=9319)	(N=1221)	(N=556)	
Modality of delivery [a]				
Spontaneous	1273 (13.7)	137 (11.2)	47 (8.5)	
Instrumental	99 (1.1)	7 (0.6)	2 (0.4)	
Elective CS	5463 (58.6)	756 (61.9)	347 (62.4)	
CS during labour	2120 (22.7)	269 (22.0)	108 (19.4)	
CS without labour	337 (3.6)	49 (4.0)	47 (8.8)	<.0001
Neonatal outcomes				
Preterm birth				
No	3618 (38.8)	378 (31.0)	91 (16.4)	
Yes	5701 (61.2)	843 (69.0)	465 (83.6)	<.001
Low birth weight				
No / No	2608 (28.0)	64 (5.2)	2 (0.4)	
Yes / No	1816 (19.5)	530 (43.4)	178 (32.0)	
Yes / Yes	4895 (52.5)	627 (51.4)	376 (67.6)	<.001
SGA				
No / No	7648 (82.1)	651 (53.3)	107 (19.2)	
Yes / No	691 (7.4)	410 (33.6)	325 (58.5)	
Yes / Yes	980 (10.5)	160 (13.1)	124 (22.3)	<.001
Low Apgar score				
No / No	8679 (93.1)	1090 (89.3)	426 (76.6)	
Yes / No	462 (5.0)	97 (7.9)	102 (18.4)	
Yes / Yes	178 (1.9)	34 (2.8)	28 (5.0)	<.001
Perinatal mortality				
No / No	9266 (99.4)	1206 (98.8)	500 (89.9)	
Yes / No	36 (0.4)	10 (0.8)	52 (9.4)	
Yes / Yes	17 (0.2)	5 (0.4)	4 (0.7)	<.001

[a] The sum did not add up to the total because of missing data. (Source: [169])

The distribution of mode of delivery and adverse neonatal outcomes by degree of weight discordance is shown in **Table 15**. Regarding the mode of delivery, caesarean section during labour and caesarean section without labour were more than 2 times more frequent in the group with BWD of 30% or more compared to the group without BWD. Preterm birth, low birth weight, small for gestational age (at least one twin), low Apgar score at 5 minutes, and perinatal mortality (at least one twin) were more frequent in twins with a discordance of 30% or more compared to those with a discordance between 20 and 29%, compared to those with a discordance of less than 20%. When

preterm births were excluded from the cohort, the positive association between adverse outcomes and BWD was still observed.
ART was inversely associated with worst outcome in both BWD twins and the control group (RR from 14% to 6% from small for gestational age to preterm). Probably due to low statistical power, no association was observed for perinatal mortality (**Table 16**).

Table 16. Relative risk (RR) and corresponding 95% confidence intervals (CI) of selected adverse outcomes according to modality of conception in the subgroups of birthweight discordance (BWD) and non-BWD. Lombardy, 2007-2021.

	Spontaneous n (%)	ART n (%)	RR (95%CI)
		BWD	
Preterm birth	678 (84.3)	252 (81.8)	0.94 (0.92-0.96)
Low birth weigh	672 (83.6)	258 (83.8)	0.94 (0.92-0.96)
Small for gestational age	420 (52.2)	153 (49.7)	0.86 (0.80-0.92)
Apgar score	123 (15.3)	35 (11.4)	0.82 (0.71-0.96)
Perinatal mortality	46 (5.7)	14 (4.6)	0.82 (0.53-1.29)
		Non-BWD	
Preterm birth	10,262 (62.7)	2826 (60.0)	0.93 (0.91-0.96)
Low birth weigh	10,418 (63.7)	2972 (63.1)	0.93 (0.91-0.96)
Small for gestational age	2660 (16.3)	721 (15.3)	0.84 (0.77-0.90)
Apgar score	798 (4.9)	185 (3.9)	0.82 (0.69-0.97)
Perinatal mortality	76 (0.5)	12 (0.3)	0.67 (0.36-1.28)

Modified from: [169]

4.2.3.3 Discussion and conclusion

In the current study, in approximately 5% of same-sex twins included in the cohort, the weight disparity between the larger and the smaller twin was 30% or more. This complication was more frequent in older women (aged 35 or more) and those who underwent ART, but less common in pluriparae. A higher proportion of adverse neonatal outcomes emerged in twins with BWD.
The discrepancy in birth weight between twins should be considered physiological up to a certain level of discordance.
Evidence suggested that older maternity age is associated with the risk of having BWD [173-175]. Given that the age of mothers at birth is increasing worldwide, especially in high-income countries, assessing the consequences of this trend is of paramount concern, since advanced maternity age is associated with increased risk of several adverse perinatal outcomes [176]. Also low educational level has been associated with BWD [177-179]. This may affect access to perinatal care, compromise management of the course of pregnancy and delay appropriate interventions, reflecting low income

and unhealthy behaviours. Other associated factors are use of ART and primiparity [174].

With regard to chorionicity, while fraternal twins have completely separate circulations in utero, about 95% of monozygotic twins have a vascular anastomosis on the single placental surface connecting the two circulations [180]. In monochorionic pregnancies, placental vascular anastomoses lead to twin-to-twin transfusion in 15% of cases, resulting in asymmetric fetal growth [181]. Conversely, monozygotic twins have also been shown to have an increased risk of BWD compared to dizygotic twins [177].

A number of limitations specific to this study need to be considered. Firstly, the data for the study are based on hospital admissions only, so there is a lack of data on clinical diagnoses made at the time of admission to hospital for childbirth or pregnancies, or which are not reported at the time of admission. Therefore, no hypotheses could be made about the pathophysiological clinical pathway leading to BWD, such as unequal distribution of the placenta, anomalies in umbilical cord insertion, placental dysfunction, and transfusion from twin to twin. We attempted to identify births where the corresponding SDO A code was reported as the principal or secondary diagnosis for such conditions, but often the underlying mechanism was not specified, even when poor fetal growth was reported. Secondly, only same-sex twins were included in the study cohort because of the known differences in birth weight and length between males and females. In this way, all the monochorionic pregnancies were included, resulting in a higher proportion than in the general population. Furthermore, the inability to distinguish dizygotic from monozygotic pregnancies was another limitation of the study.

4.2.4 VI Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Parazzini F, Viganò P, et al. Probability of second live birth after first natural and medically assisted reproduction-mediated live birth: a historical cohort study. Acta Obstet Gynecol Scand. 2023;00:1-8. doi:10.1111/aogs.14685 [182]**

4.2.4.1 Specific aim and methods

Infertility is generally defined as the incapacity to have a child, but according to a more appropriate definition states that it is the incapacity to have the desired number of children. A challenging issue to investigate is the role of ART in fulfilling couples' reproductive intentions field of the ART, family rate is a neglected but emerging issue. The aim of this study was to assess the probability of having a second live birth according to the mode of conception of the first birth. The focus was on the role of ART use in second births.

All women hospitalised for delivery between 2007 and 2017 were included and followed-up untill 2021.

Descriptive statistics were used to summarise women's characteristics at the time of their first live birth, and differences in observed variables between women with one birth and women with two births were tested using Chi-squared.
The Kaplan-Meier method was used to estimate the overall cumulative probability of a second live birth in women who had a first naturally conceived birth and in women who had a first ART-mediated birth. To estimate the hazard ratio (HR) and 95%CI of the association between mode of conception at first birth and the probability of having a second birth, Cox proportional hazards models were fitted. The models were adjusted for the mother's age and level of education.
Analyses were repeated and stratified by maternal age (i.e. <35, ≥35 years) according to the mode of conception of the second birth.
Each woman accumulated person-years of follow-up from the first birth until the earliest of the following dates: the date of the second birth, the date up to which the mother was covered by the regional health care system, the date of the mother's death, and 31 December 2021. Mothers were considered right censored if they moved out of the region, died, or did not have a second birth by the end of follow-up.

4.2.4.2 Results

431,333 natural births and 16,837 ART-mediated births were available for the analysis. The probability of having a second birth was 58.6% in the group of women who had their first birth after natural conception. This was 32.1% (HR=0.68, 95%CI:0.66-0.70) for women who conceived after ART (**Figure 6**). For women who conceived their first child naturally and after ART, the median time to a second birth was 3.2 and 2.8 years, respectively (p-value<0.001).

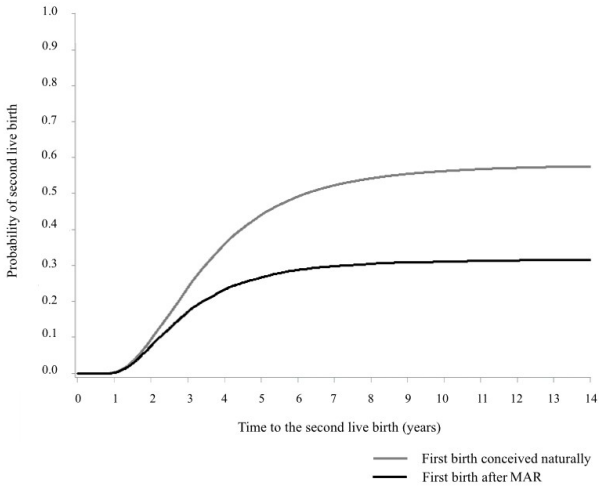

Figure 6. Probability of having a second live birth according to the modality of conception of the first. Lombardy, 2007-2021. (Source: [182])

The probabilities of having a second live birth after MAR and natural conception were 1.1% and 59.3%, respectively, in women who had a first live birth by natural conception. For women who had undergone ART to achieve their first live birth, the probabilities were 11.5% (HR=15.12, 95%CI: 13.99-16.35) and 25.2% (HR=0.52, 95%CI:0.50-0.54), respectively (**Figure 7**).

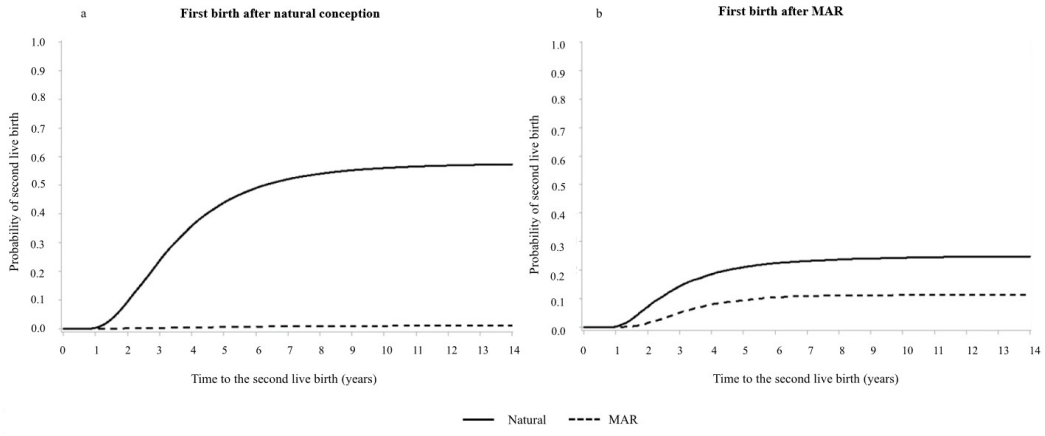

Figure 7. Probability of having a second natural or MAR-mediated live birth according to the modality of conception of the first. Lombardy, 2007-2021. (Source: [182])

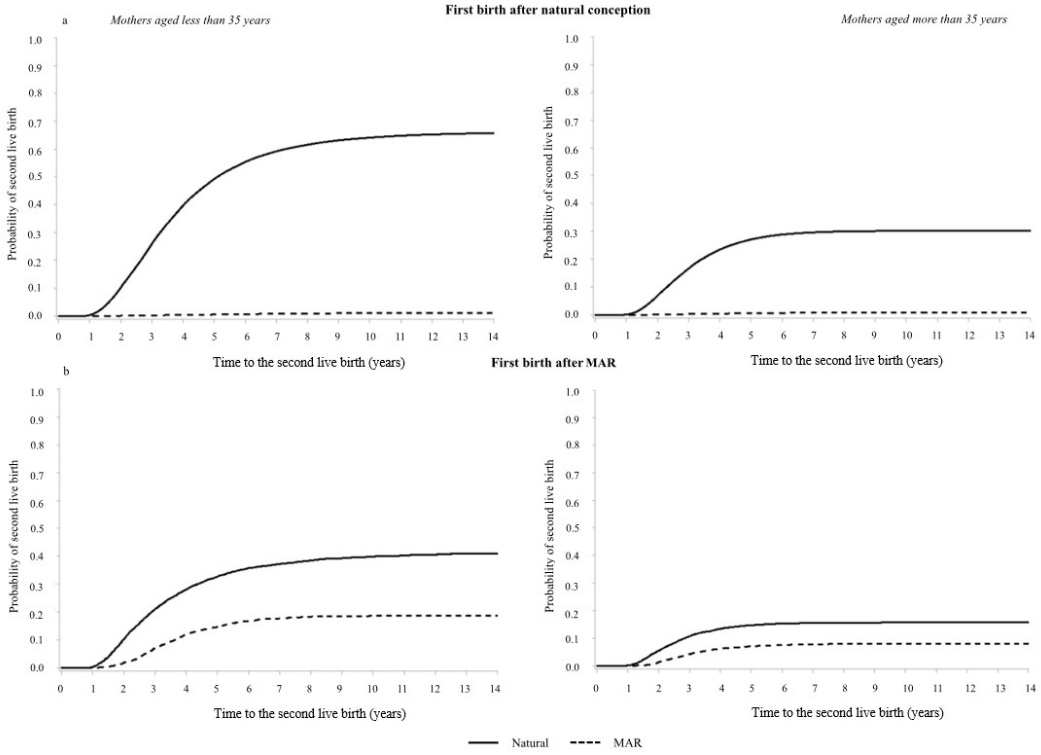

Figure 8. Probability of having a second natural or MAR-mediated live birth according to the modality of conception of the first and maternal age. Lombardy, 2007-2021. (Source: [182])

Compared with the group of women who had their first birth after natural conception, the probability of a second natural birth was significantly reduced for both age subsets (HR=0.58, 95%CI:0.55-0.60 and HR=0.62, 95%CI:0.59-0. 66 for women aged less than 35 years and those aged 35 years and older, respectively) and the probability of a second birth after ART were significantly increased in the group of women who achieved their first MAR-mediated birth (HR=19.49, 95%CI:17.66-21.50 and HR=10.86, 95%CI:9.70-12.15 for women aged less than 35 years and those aged 35 years and older, respectively) (**Figure 8**).

4.2.4.3 Discussion and conclusion

According to the study, the probability of a second birth after a first one achieved by MAR was about half that of mothers who conceived their first child naturally, at about 30% and 60% respectively. In terms of second live births after MAR, one in ten women who had a first live birth after MAR had a second baby after MAR. When only women who had a first live birth naturally were considered, this proportion is drastically lower (about 1%). The interpretation of the results must take into account that couples who used MAR for the first birth were not directly comparable with the general population, as they were by definition less fertile. Therefore, the focus was on the mode of conception of second births according to the use or non-use of MAR for the first birth.

Regardless of the method of conception, younger women were more likely to have a second baby. This is not surprising: age is known to play an important role in predicting the probability of conceiving naturally, and also of conceiving through MAR [183-185]. However, in a study looking at the occurrence of natural conception in patients who had discontinued unsuccessful ICSI treatment, maternal age did not affect the chance of conception [186].

In the study, according to previous evidence [187,188], MAR makes a limited contribution to achieving the desired family size. Second births were more likely to occur naturally than through MAR also in the group of women who had their first birth after MAR.

Approximately one in five couples - particularly those who are younger, those who have been infertile for a shorter period of time, or those who have unexplained infertility - report a natural pregnancy after a first successful MAR attempt [183,184].

The early use of MAR, especially in cases of unexplained infertility, and the positive placebo effect [189], but also psychological aspects [190], may explain this high rate of natural conception after MAR attempts.

It is not possible to disentangle from this study the reasons for the low rate of MAR use for conception of the second child. Only part of this phenomenon can be explained by the observation that a substantial proportion of women, who previously underwent MAR, conceived naturally. However, this proportion is smaller than the rate of natur al second pregnancies observed among fertile women of childbearing age, which suggests that a consistent proportion of women fail to achieve the most frequently intended number of children, that is, two. This may be an indication of unmet need. Compared to the general population, couples who have faced and overcome the difficulties of MAR for their first conception may not be less interested in having a second child. However, we believe that concerns about the safety, logistical, emotional, and psychological burden of MAR may play a crucial role. The overwhelming journey of MAR may be less likely for infertile women with one child. This is also likely to be true for women who have conceived naturally. This is evidenced by the extremely low rate of MAR uptake among women who have had a first natural conception. It is not likely that the reduced number of second births is due to a reduced success rate of the treatment, since a first pregnancy with MAR is a positive predictor of a second success [191]. In addition, we did not expect financial reasons to play a major role, as MAR is provided free of charge by the public health system in the study area.

A number of limitations of the study need to be recognised. First of all, we were not able to determine the proportion of women who underwent MAR and did not succeed. For this reason, it was not possible to deduce from our data the rate of return of women who had undergone MAR for a previous birth, nor was it possible to assess the number of failed attempts in women who went on to conceive naturally. Also, whether women changed partners between the first and second child was not known. This may have been a confounding factor, albeit with a plausibly small role in the explanation of the significantly lower rate of second births with MAR.

4.2.5 VII Study

The content covered in this chapter refers to a submitted paper:

> Long-term impact of COVID-19 pandemic on ART-mediated births in Lombardy, Italy.

4.2.5.1 Specific aim and methods

During the early phase of the COVID-19 pandemic, the recommendations by scientific societies to postpone the initiation of new fertility treatments [192] caused a rapid and drastic decline of ART-mediated births. The concerns about hospital infection could be expected to be tempered during the subsequent waves of the COVID-19 pandemic when restrictions were also less strict. However, the general burden on the health systems was still remarkable and a detrimental effect on ART provision remained, at least for Centers performing within the public health system. Moreover, couples may have decided on their own to postpone ART conceptions and childbirth also during the subsequent waves due to pandemic-related uncertainty and financial instability, travel restrictions, and anxiety about the harmful effects of SARS-CoV-2 infection during pregnancy. On the other hand, after the first months of the pandemic, ART clinics implemented more effective organizational measures to recover the activity.

ART-mediated births with hospitalization from 1st January 2021 to 31st December 2022 were identified. Data relating to the two-year period 2019-2020 were retrieved from a previous investigation [168]. The absolute number of ART-mediated births per month was considered and the percentage variation in relation to the prepandemic period, i.e., 2019, was computed. ART-mediated births were obtained after ovarian stimulation, IUI, conventional IVF, or ICSI.

4.2.5.2 Results

Figure 9 shows the number of ART-mediated births per month during the period between 2020 and 2022 and the percentage variation compared to the same months of 2019.
A substantial decrease in ART-mediated births emerged in December 2020, January 2021, and February 2021, with a drop of -86.6%, -99.2%, and -57.8%, respectively. Subsequently, the situation rapidly recovered to 2019 levels. From March to December 2021 the number of ART-mediated births was similar to that observed in the same period in 2019: more births were observed in April, May, September, and December and less in the remaining 6 months. During 2022, the absolute number of ART-mediated births surpassed those observed in the prepandemic period in all months

except for January and October. The maximal increase was observed in August (+33.0%).

When we considered the total number of ART-mediated births per calendar year, a substantial reduction was observed in 2020 (-10.5%, 95%CI: -15.2% to -5.9%) and in 2021 (-16.7%, 95%CI: -15.2% to -5.9%) compared to 2019, while an increment emerged in 2022 (+13.8%, 95%CI: + 8.3% to +19.4%).

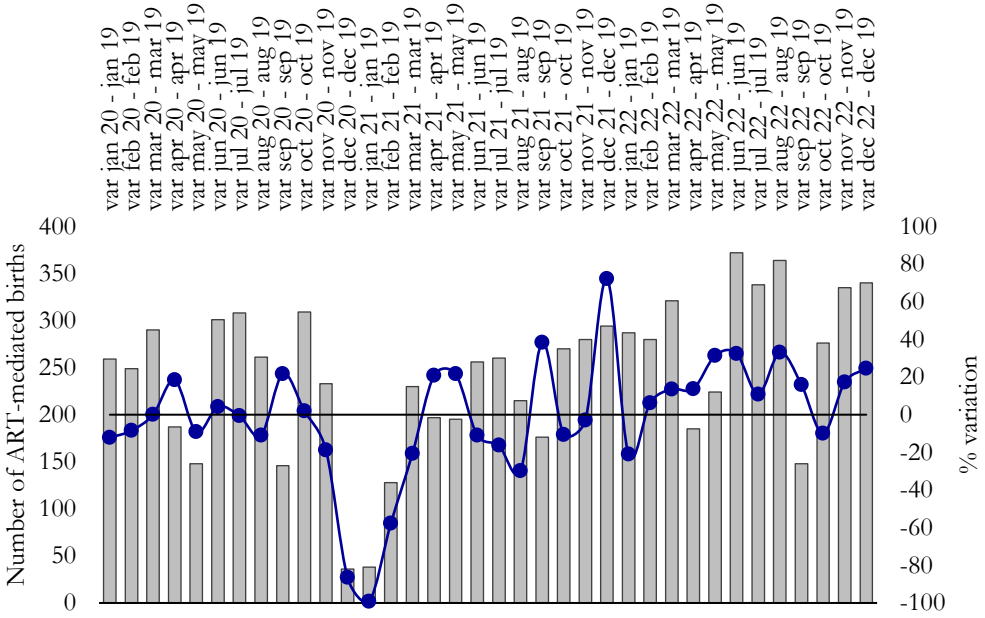

Figure 9. Number of assisted reproductive technology (ART)-mediated births per month and percentage variation compared to the same month of 2019.

4.2.5.3 Discussion and conclusion

The results of the analysis show that the restrictions due to the COVID-19 pandemic had a marked effect on the number of ART-mediated births; an overall reduction of ART-mediated births of about 11% and 17% respectively in 2020 and 2021 was observed in comparison with numbers of ART-mediated births in 2019. Instead, during 2022, an increase of approximately 14% in ART-mediated births emerged.

In Lombardy, two lockdown periods were imposed by the National and Regional Authorities to face the COVID-19 pandemic: during the first (from March to June 2020), ART clinics were closed, while in the second (from November 2020 to April 2021) ART clinics could open but practical restrictions to limit infection spread were in place and limited the possibility to perform ART cycles. Moreover, activity was restrained in Centers that were part of general hospitals because of the need to divert healthcare personnel in COVID-19 related activities.

On the other hand, the increase registered in 2022 was probably due to the effort made by ART clinics to recover couples who had delayed procedures, even if it must be

underlined that a trend toward an increase of ART-mediated births were present in Lombardy also before the pandemic [147].

In the interpretation of these findings, we must note that Italy has been characterized by a sharp decline in natality for a long time, even preceding the COVID-19 outbreak [168]. Therefore, the employment of the year 2019 as a comparator may overestimate the detrimental effects. For the same reason, it cannot be accurately estimated whether the recovers observed in 2022 fully compensated for the missed births of 2020 and 2021.

In conclusion, the COVID-19 outbreak had an initial devastating impact on ART-mediated births. However, the system positively and rapidly reacted and presumably effectively compensated.

4.3 Pharmacoepidemiology in pregnancy

4.3.1 VIII Study

The content covered in this chapter refers to a published paper:

> **Esposito G, Cantarutti A, Franchi M, Corrao G, Parazzini F. Drug Prescriptions during Pregnancy in Lombardy: Temporal Trends and the Impact of the Onset of the COVID-19 Pandemic. Pharmacoepidemiology. 2023;2(3):249-256. https://doi.org/10.3390/pharma2030021 [193]**

4.3.1.1 Specific aim and methods

Understanding the rational use of medications in pregnancy and exploring patterns of use is essential, and monitoring medication prescribing patterns during pregnancy could be an invaluable tool for assessing compliance with recommended supplement and medication use in clinical practice. By studying these patterns, we can further refine guidelines and recommendations to optimise the wellbeing of both the mother and the developing fetus.
Hence, the main objective of the present study is to provide a comprehensive overview of the prevalence of dispensed drug prescriptions throughout the course of pregnancy, encompassing various therapeutic categories. Furthermore, in recognition of the transformative impact of the COVID-19 pandemic, its potential influence on prescription patterns will also be taken into consideration.

The study cohort consisted of all births occured between 2010 and 2020.
The outpatient drug prescriptions registry was used to identified dispensed drug prescriptions. Drugs were classified according to the WHO ATC coding system. Anatomical subgroups, chemical subgroups, and single active agents were considered.

The prevalence of drug prescrptions was determined by calculating the proportion of pregnant women who received at least one prescription out of the total number of births. Pregnancy was considered overall and also by trimesters, which were calculated from the date of conception at three months in three months. Exposure in each trimester of pregnancy was defined by the presence of at least one prescription in the three periods of interest. However, if women had only one prescription in the first trimester and none in the second and third, this was considered as exposure in the first trimester only. When considering single active agents, the prevalence of drug prescriptions was provided for selected periods (i.e., 2010 to 2012, 2013 to 2015, 2016

to 2018, and 2019 to 2020). For the 2016 to 2018 period, a comparison with the AIFA national report was proposed.
In addition, the use of antibiotics (ATC: J01) was deeply investigated. The prescription incidence rate was defined as the number of antibiotics prescriptions per 1000 person-time, calculated from the date of conception until the date of delivery. An interrupted time series analysis was performed to compare antibiotic prescriptions trend, taking into account the seasonality of infections, between the period January 2010 and February 2020 and the period between April 2020 and December 2020. The choice of the periods was made to consider the impact of COVID-19 pandemic.

4.3.1.2 Results

Of the 780,075 women in the cohort, 497,515 (63.8%) used at least one medication during pregnancy, including minerals and vitamins: 310,233 (39.8%) in the first trimester, 243,160 (31.2%) in the second trimester and 273,563 (35.1%) in the third trimester. If vitamins and minerals are excluded, 373,075 (47.8%) women had at least one prescription, the proportions by trimester being 31.1%, 26.0%, 64 and 27.2% in the first, second and third trimesters respectively.

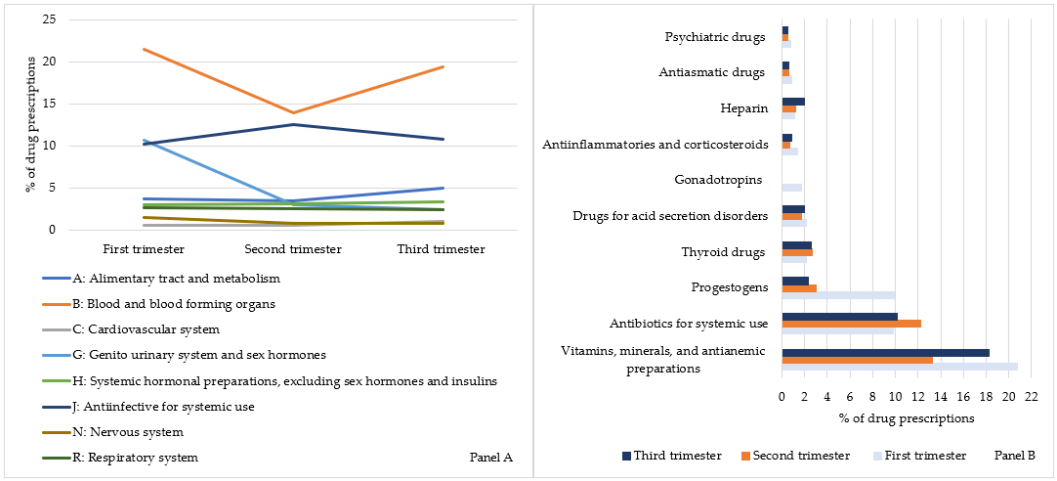

Figure 10. Prevalence of drug dispensing (%) according to selected anatomical (Panel A) and chemical groups (Panel B) across trimesters of pregnancy. Lombardy, 2010-2020. (Source: [193])

Figure 10 shows the prevalence of drug prescriptions in the three trimesters according to selected anatomical (Panel A) and chemical (Panel B) subgroups. On the basis of anatomical group classes, drugs for diseases of the blood and haematopoietic organs (ATC B) were observed in the highest proportion of women in all trimesters of pregnancy (21.5%, 13.9% and 19.4% in the first, second and third trimesters respectively). Antimicrobials for systemic use (ATC J) were the second most commonly prescribed drugs during pregnancy, with a peak of 12.5% in the second trimester. In terms of chemical classification, vitamins, minerals and anti-anaemic

agents were prescribed in 20.8%, 13.3% and 18.3% of deliveries, respectively, during the different trimesters of pregnancy.

Among the most commonly prescribed single agents, folic acid ranked first with about 1 in 4 women, followed by iron supplements and progestogens (**Table 19**). There was an increase from 9.2% in 2010-2012 to 14.4% in 2019-2020 in the proportion of births for which a progestogen was prescribed. Amoxicillin (with or without clavulanic acid), fosfomycin, azithromycin, clarithromycin and cefixime were the most commonly prescribed antibiotics.

Table 19. The most prescribed drugs. Lombardy, 2010-2020.

Drug (ATC)	2010-2012 n (%)	2013-2015 n (%)	2016-2018 n (%)	2019-2020 n (%)
folic acid (B03BB01)	41,804 (18.0)	52,527 (23.6)	54,419 (26.6)	30,741 (25.2)
ferrous solfate (B03AA07)	40,590 (17.5)	42,874 (19.3)	40,473 (19.8)	24,370 (20.0)
progestogen (G03DA04)	21,353 (9.2)	28,386 (12.8)	29,848 (14.6)	17,532 (14.4)
amoxicillin/clavulanic acid (J01CR02)	26,411 (11.4)	26,646 (12.0)	23,257 (11.4)	12,953 (10.6)
amoxicillin (J01CA04)	17,219 (7.4)	15,878 (7.1)	12,384 (6.1)	6144 (5.0)
fosfomycin (J01XX01)	11,155 (4.8)	11,674 (5.3)	11,010 (5.4)	6397 (5.2)
levothyroxine sodium (H03AA01)	7961 (3.4)	8817 (4.0)	8030 (3.9)	5027 (4.1)
azithromycin (J01FA10)	7730 (3.3)	8775 (4.0)	5927 (2.9)	2712 (2.2)
beclometasone (R03BA01)	5996 (2.6)	6615 (3.0)	5543 (2.7)	2490 (2.0)
enoxaparin (B01AB05)	3697 (1.6)	4109 (1.8)	4011 (2.0)	2720 (2.2)
colecalciferol (A11CC05)	231 (0.1)	1660 (0.7)	5723 (2.8)	6444 (5.3)
alginic acid (A02BX13)	4017 (1.7)	4441 (2.0)	3651 (1.8)	1858 (1.5)
ferrous glycine sulfate (B03AA01)	3897 (1.7)	2717 (1.2)	2475 (1.2)	1720 (1.4)
estradiol (G03CA03)	1681 (0.7)	2707 (1.2)	3298 (1.6)	1704 (1.4)
salbutamol (R03AC02)	2908 (1.3)	2808 (1.3)	2388 (1.2)	1196 (1.0)
clarithromycin (J01FA09)	2724 (1.2)	2605 (1.2)	2292 (1.1)	1204 (1.0)
cefixime (J01DD08)	1866 (0.8)	2201 (1.0)	2525 (1.2)	1883 (1.5)

Source: [193]

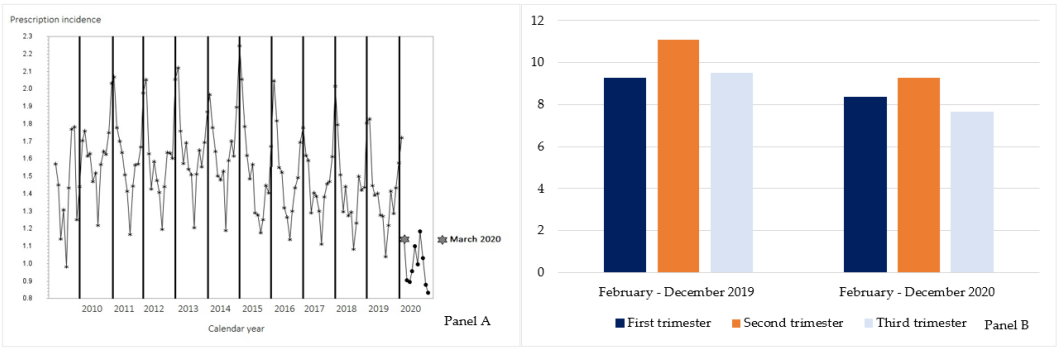

Figure 11. Trend in antibiotics use (Panel A) and antibiotics use in 2019-2020 according to trimesters of pregnacy (Panel B). Lombardy, 2010-2020. (Source: [193])

Focusing on antibiotics, the prescription rate decreased in all three trimesters between February 2020 and December 2020, the first period of COVID-19 pandemic spread,

to 8.3%, 9.3% and 7.6%, respectively, compared to 9.3%, 11.1% and 9.5% in the same period of the previous year (**Figure 11**, panel A). Among women who were pregnant between April 2009 and February 2020, there was a decreasing trend in antibiotic dispensing (p-value=0.04) (**Figure 11**, panel B). After controlling for this trend, there was a significant further decrease of about 0.54% after COVID-19 spread in March 2020. In the following months, however, the trend was significantly different from the previous years (p-value<.01). This describes an increasing trend (p-value<.01).

4.3.1.3 Discussion and conclusion

Despite concerns about the issue, medication use during pregnancy is a common practice. Evidence suggests that up to 27-99% of pregnant women take at least one drug, including vitamin and mineral supplements [74]. The findings of the study in exam were consistent with literature. The prevalence of dispensed drug prescriptions during pregnancy was about 48%. When vitamin and mineral supplements were included, the prevalence was just under 65%. The most commonly prescribed drugs were folic acid and iron preparations, followed by progestogen and antibiotics. Throughout the study period, there was an increasing trend in the dispensed prescriptions for progestogen and a decreasing trend for antibiotics.
In particular, the use of folic acid, progesterone and antibiotics is discussed below.
Both national and international guidelines recommend that women who want to become pregnant take 0.4 mg of folic acid daily, starting at least one month before conception and continuing until the twelfth week of pregnancy. This is to help prevent neural tube defects and other congenital anomalies [194]. In Italy, official recommendations were introduced in April 2004 and folic acid has been freely available on prescription since 2005. However, there is insufficient awareness of the benefits of the vitamin among women planning a pregnancy, and access to preconception services remains limited [195]. In the study described, given the administrative nature of data, the actual consumption of folic acid was probably underestimated due to the high use of over the counter drugs [196], being stable around 25% after 2016.
In pregnancy, the use of progestogen has attracted attention and interest for its potential benefits. Its use is a subject of debate and research in the medical community. While progestogen is primarily known for its essential role in supporting pregnancy by maintaining the uterine lining and preventing its shedding, no evidence exists to substantiate the prolonged administration of progestogens during pregnancy with the goal of enhancing the occurrence of full-term births and/or increasing live birth rates in women who experience threatened or recurrent pregnancy loss [197,198]. In addition, nowadays, progesterone plays a crucial role in ART, particularly in supporting the luteal phase and ensuring a favourable uterine environment for the embryo [199]. In line with this, the study under consideration observed an increasing trend in the use of progestogen during pregnancy.

In pregnancy, one of the most commonly used classes of drugs is antibiotics; approximately one out of every three women takes them [111,200,201]. According to this as just said, antibiotics represented one of the most prescribed drugs in the investigation in exam. A reduction in the use of antibiotcs emerged during the whole study period, especially in the early phase after the onset of the COVID-19 pandemic, probably due to the reduction of respiratory tract infections thanks to the preventive measure of lockdown. However, after March 2020, an increasing trend was observed.
It should be remembered that, due to the administrative nature of the data, only drug prescriptions dispensed and reimbursed by the NHS were included; therefore, the actual use of drugs may be underestimated, excluding non-reimbursed and over the counter drugs.
In conclusion, monitoring medication use during pregnancy is of paramount importance for several reasons. First, it helps to ensure the safety and health of both the mother and the fetus, as some medications may carry known or unknown risks during pregnancy. Secondly, it allows assessment of whether clinical practice is following recommended guidelines for the use of supplements and medications, thereby ensuring improvement in the quality of prenatal care. Monitoring also provides valuable data for research and policy-making to further improve maternal and foetal health outcomes.

4.3.2 IX Study

The content covered in this chapter refers to a submitted paper:

> Does preterm birth increase the initiation of antidepressants use during the post-partum? A population-based investigation

4.3.2.1 Specific aim and methods

Perinatal depression and anxiety affect a significant number of women, and the stress associated with having a preterm baby can exacerbate these symptoms. These conditions can be prolonged and severe, with debilitating and potentially fatal consequences for mothers, couples and children. In some more severe cases or those that cannot be treated with psychotherapy, antidepressant treatment is necessary. Antidepressant initiation during postpartum can be proxy of depression, but also of another mental illess such as anxiety, or even bulimia nervosa.
The primary objective of the present study was to investigate the potential association between the occurence of preterm birth and the use of antidepressants during the year following childbirth, while also considering various demographic and medical factors that might influence this relationship.

The study cohort included women who gave birth between 2010 and 2020, excluding those with a positive history of depression or anxiety.

Exposure. Preterm birth was defined as any birth after 22 weeks of gestation and before 37 weeks [149]. Preterm birth could be categorised according to gestational week, i.e. extremely preterm (<28 weeks), very preterm (28-31 weeks), moderate to late preterm (32-36 weeks). In the analysis, the first two categories were combined into one, as births before 28 weeks represent less than 5% of all births before 37 weeks.
Outcome. The initiation of antidepressants use in the year after birth (time of follow-up) was considered having at least a dispensed drug prescription for antidepressant medications (ATC code: N06A).

The rate of antidepressant prescriptions per 100 according to selected maternal socio-demographic and clinical characteristics (i.e. age, nationality, educational level, parity, and diabetes) and current obstetric anamnesis (i.e. mode of conception, mode and timing of delivery) was computed. Log-binomial regression models were fitted to estimate the RR and the corresponding 95%CI of use of antidepressants according to these covariates.
Further, multivariate log-binomial regression model was fitted to estimate the relative risk (RR) and the corresponding 95%CI of use of antidepressants according to gestational weeks at birth, accounting for potential confounders, such as maternal age at birth (<30, 30-34, 35-39, >39 years), nationality (Italian or not), educational level

(university, high school, middle or primary school), parity (nulliparae or pluriparae), mode of conception (natural or medical assisted), and diabetes (i.e., at least a prescription of an antidiabetic drug during pregnancy considering both gestational and pre-existing diabetes).
Women with only one antidepressant prescription in the postpartum year were excluded as a sensitivity analysis. Stratified analyses were also performed by maternal age, nationality, educational level, parity and mode of pregnancy.

4.3.2.2 Results

During the study period, 727,850 deliveries were identified, 689,396 (94.7%) at term and 38,454 (5.3%) preterm. During the year after birth, 528 women with preterm birth (1.37 per 100 births) and 6026 (0.87 per 100 births) with term birth had at least a filled prescription of antidepressant drugs.

Table 17. Relative risk (RR) and corresponding 95% confidence intervals (CI) of initiation of antidepressant use according to selected covariates. Lombardy, 2010-2020.

Maternal characteristics	**Reference population**		**Antidepressant users**		**Rate per 100**	**RR[a]**	**95%CI**
	N	**%**	**N**	**%**			
Maternal age (years)							
<30	200,497	27.8	1584	24.2	0.78	1.00[b]	Ref.
30-34	256,694	35.6	2224	33.9	0.86	1.10	1.03-1.17
35-39	203,929	28.3	2023	30.9	0.98	1.25	1.17-1.34
>39	60,176	8.3	723	11.0	1.20	1.51	1.39-1.65
Nationality							
Italian	551,592	76.5	5463	83.4	0.98	1.00[b]	Ref.
Not italian	169,704	23.5	1091	16.7	0.64	0.65	0.61-0.69
Educational level							
University	235,181	29.5	1929	29.5	0.81	1.00[b]	Ref.
High school	314,868	44.9	2933	44.9	0.92	1.13	1.07-1.20
Middle/primary school	168,721	23.5	1673	25.6	0.98	1.21	1.13-1.29
Diabetes							
No	710,110	98.5	6436	98.2	0.90	1.00[b]	Ref.
Yes	11,186	1.5	118	1.80	1.04	1.16	0.97.1.39
Parity							
Nulliparae	352,857	48.8	3252	49.6	0.92	1.00[b]	Ref.
Pluriparae	369,439	51.2	3302	50.4	0.89	0.97	0.92-1.02
ART							
No	697,694	97.1	6281	96.3	0.89	1.00[b]	Ref.
Yes	20,749	2.9	242	3.7	1.15	1.29	1.14-1.47
Modality of delivery							
Vaginal	533,729	74.1	4395	67.2	0.83	1.00[b]	Ref.

Cesarean section	186,241	25.9	2143	32.8	1.14	1.39	1.32-1.47
Gestational age at birth (weeks)							
≥37	683,370	94.7	6026	91.9	0.87	1.00[b]	Ref.
32-36	33,635	4.7	448	6.8	1.31	1.50	1.37-1.65
<32	4291	0.6	80	1.2	1.83	2.09	1.68-2.61

[a] Estimated from log-binomial regression model. [b] Reference category.

Table 17 provides the crude RR of initiation of antidepressant use according to selected covariates. The risk was increased for increasing age (RR=1.51, 95%CI:1.39-1.65 for women aged 39 years or more versus women aged less than 30 years), lower educational level (RR=1.21, 95%CI:1.13-1.29 for women who attended middle/primary school versus women who attended university), medically assisted conception (RR=1.29, 95%CI:1.14-1.47), cesarean section (RR=1.39, 95%CI:1.32-1.47), diabetes (RR=1.16, 95%CI:0.97-1.39), and preterm birth (RR=1.50, 95%CI:1.37-1.65 for births between 32 to 36 weeks and RR=2.09, 95%CI:1.69-2.61 for deliveries before the 32 complete weeks). The risk was decreased in not Italian women (RR=0.65, 95%CI:0.61-0.69). No differences emerged for parity (RR=0.97, 95%CI:0.92-1.02).

Table 18. Relative risk (RR) and corresponding 95% confidence intervals (CI) of initiation of antidepressant use according to timing of birth. Lombardy, 2010-2020.

Gestational age at birth (weeks)	Antidepressant users, N (%)	Non users, N (%)	RR[a]	95%CI
		Overall		
≥37	6026 (91.9)	683,370 (94.7)	1.00[b]	(Ref.)
32-36	448 (6.8)	33,635 (4.7)	1.38	1.26-1.53
<32	80 (1.2)	4291 (0.6)	1.83	1.47-2.29
		Vaginal delivery		
≥37	4184 (95.2)	514,648 (96.4)	1.00[b]	(Ref.)
32-36	189 (4.3)	17,761 (3.3)	1.29	1.12-1.49
<32	22 (0.5)	1320 (0.3)	2.05	1.35-3.11
		Cesarean section		
≥37	1831 (85.4)	167,588 (90.0)	1.00[b]	(Ref.)
32-36	255 (11.9)	15,739 (8.5)	1.47	1.29-1.68
<32	57 (2.7)	2914 (1.6)	1.77	1.36-2.31

[a] Estimated from log-binomial regression model including terms for maternal age at birth, nationality, educational level, parity, modality of conception, mode of delivery, and diabetes. [b] Reference category.

Table 18 shows the RR of antidepressants' use according to the timing of birth adjusted for selected confounders. Preterm births were related to a 38% increased risk of maternal use of antidepressants in the year after birth (adjusted RR=1.38; 95% CI: 1.26-1.53) for moderate to late preterm births and 83% increased risk (adjusted RR=1.83; 1.47-2.29) for extremely and very preterm births. Excluding women who

had only one antidepressant prescription, a similar association was observed (adjusted RR=1.41, 95%CI: 1.23-1.62 in moderate to late preterm birth and adjusted RR=1.81, 95% CI: 1.31-2.50 in extremely and very preterm births).
The association was observed in all the subgroups analyzed (**Figure 12**).

Selected strata	RR*	95%CI	
Maternal age (years)			
<30	1.50	1.25	1.82
30-34	1.41	1.20	1.66
35-39	1.41	1.20	1.65
>39	1.46	1.15	1.85
Nationality			
Italian	1.42	1.29	1.57
Not italian	1.54	1.25	1.89
Educational level			
University	1.40	1.17	1.67
High school	1.51	1.33	1.73
Middle/primary school	1.35	1.14	1.61
Parity			
Nulliparae	1.51	1.33	1.70
Pluriparae	1.37	1.20	1.58
ART			
No	1.45	1.32	1.59
Yes	1.46	1.01	2.10

Figure 12. Relative Risk (RR) and corresponding 95% confidence interval (CI) of anxiety/depressive disorders in the year after the birth in women who had a preterm birth in strata of selective covariates. Lombardy, 2010-2020.

* Estimated from log-binomial regression model including terms for maternal age at birth, nationality, educational attainment, parity, modality of conception, mode of delivery, and diabetes; unless the variable was the stratification factor.

4.3.2.3 Discussion and conclusion

In the investigation, mothers who had a preterm birth had an excess risk of starting antidepressant treatment in the year after the birth of about 80%.
Selective serotonin reuptake inhibitors are the mainstay of drug treatment for perinatal depression, and fluvoxamine, paroxetine and sertraline are preferred in lactating women, resulting in the lowest serum drug levels in infants [106]. It's worth noting that treatment approaches for postpartum depression vary around the world; for example, while in the USA about half of women diagnosed with postpartum depression were on antidepressants [108], European countries tend to have lower levels of antidepressant use [105]. This discrepancy, possibly due to differences in medical practice and cultural norms, raises awareness of the potential undertreatment of women with new-onset postpartum depression in Italy. In the current investigation almost 1% of women of the cohort had at least a prescription for antidepressant drugs during the year after birth.

According to a systematic review [202], mothers of preterm infants were at a high risk of depression in the immediate postpartum period compared with mothers of infants born at term. Several reasons could be taken into consideration.
First, there is an inverse relationship between gestational age and comorbidity, length of hospital stay and neonatal management difficulties [202]; in line with this, a study, including parents with an infant admitted to a neonatal intensive care unit, suggested that the shorter the infant's week, the higher the mothers' depression scores [203]. In contrast, another study found no association between duration of parent-child closeness during the first weeks of hospitalisation and parental depressive symptoms [204].
Another reason could be found in the inadeguate bonding and attachment between mother and infant [202]. Poor infant engagement and orientation, typical of preterm babies, has been shown to have a negative impact on a mother's feelings towards her infant. A causal relationship between perinatal mood disorders in mothers and challenging child outcomes may run both ways. It has been reported that poor mental health affects a mother's ability and quality of bonding and interaction with her infant, as infant temperamental difficulties predispose to altered maternal mood [205]. This is an area of public health priority, as early maternal and neonatal attachment strongly influences future health outcomes and emotional, social and cognitive development.
A common risk factor for both conditions, sleep disturbance, may also explain the link between mental disorders and preterm birth [206-209].
A substantial limitation should be considered; women who took antidepressants could be taking them for depression, but also for a range of other conditions. Therefore, the use of antidepressant could not be an accurate proxy for postpartum depression.
This research highlights the urgent need for evidence-based approaches to improve care, not neglecting mental health in high-risk pregnanc

CHAPTER 5 - DISCUSSION

The core focus of the research projects reported in this thesis was to explore and investigate the growing concerns about reproduction and childbearing health.

Over the past thirty years, particularly in high income countries, reproduction has undergone significant changes, influenced by a combination of social, economic, technological, and cultural factors. This has meant moving away from traditional patterns of reproduction and adopting new approaches and perspectives towards family planning and childbearing. One of the most substantial changes has been the delay in the age at which individuals and couples decide to have children. In the past, people used to start families when they were younger, but in recent decades many people in modern society have decided to delay having children to pursue education, career opportunities, or personal goals.
Advanced maternal age has been linked to a higher incidence of comorbidities during pregnancy and childbirth; older mothers are more likely to experience both pregnancy complications and coexisting morbidities during pregnancy. These health challenges can potentially impact both the well-being of the mothers and the newborns.
Delaying childbearing can also contribute to an increase in fertility problems. Female fertility declines significantly with age, especially after the age of 35, due to a decrease in the quantity and quality of oocytes. In this perspective, advances in reproductive technologies have also been crucial in changing reproductive patterns. For individuals and couples facing infertility problems, assisted reproduction programmes have provided options. Moreover, reproductive technologies have also enabled people to conceive and have children later in life, contributing to the trend towards delayed childbearing.

In this framework, monitoring of reproductive health is of paramount importance and addressing these clinical and sociological challenges necessitates comprehensive healthcare and public policy efforts. It is essential to recognise the changing dynamics of family planning and to adapt health systems and clinical practices to ensure reproductive health. In Italy, CedAP is the richest source of health, epidemiological, and socio-demographic information related to the birth event, representing an essential tool for national and regional health planning in the maternal and child area.
The availability of high-quality integrated individual data from NHS outpatient and inpatient claims databases and the record linkage process provides the opportunity to track and evaluate the full care pathway. In addition, the systematic collection of administrative data allows for the timely detection of trends, patterns, and potential issues in childbirth practices and outcomes. In this view, real-world evidence refers to data and information collected from routine clinical practice and the healthcare environment, including data from electronic health records, including healthcare utilization databases and claims and billing data, that reflect the realities of patient care and outcomes in real-world settings. However, as these databases are collected for purposes such as payment and reimbursement and not designed for clinical research, the clinical content of the administrative data may only include the demographic characteristics and diagnoses of the patients and the medical codes for the procedures

performed, and may not be accurately captured. Moreover, confounding variables, such as lifestyle factors (e.g., smoking, alcohol use, obesity), are unknown in administrative databases.

The first topic addressed in this thesis was PAC. As mentioned above, the management of cancer during pregnancy presents unique challenges due to clinical, as well as human and social, concerns about the impact of cancer on the developing fetus.
A first study aimed to assess the association between a diagnosis of PAC and adverse perinatal outcomes, found that oncological women tended to have a higher incidence of iatrogenic preterm birth. This suggests that medical interventions, that may be required by the presence of cancer during pregnancy, lead to an increased probability of iatrogenic births also before the term of pregnancy. The need for timely and appropriate medical interventions to manage both the cancer and the pregnancy can sometimes lead to induced preterm birth, which is an important consideration in the overall care and management of these individuals. However, a specific oncological indication may not always exist and it is imperative that iatrogenic preterm birth is kept to a minimum, an action that should be taken with caution given the delicate balance between the health of the mother and the fetus.
The study investigating the impact of a cancer diagnosis on pregnancy outcome has shown a downward trend in abortions in favour of live births. Recent studies suggest that advances in research and clinical practice, together with increased awareness of the potential for treating oncological conditions in pregnant women, have contributed to this trend.

ART was the second topic approached in the thesis, from both clinical and sociological point of view. Understanding the profound influence of infertility on the quality of life and overall well-being of individuals, it is crucial to address the challenges associated with this condition. In recent years, remarkable advances in ART have revolutionised the field of fertility treatment. These advances also highlight the importance of continued investment in research, accessibility, and affordability to ensure that a wider population can benefit from these advances in reproductive healthcare.
Some reported studies aimed to investigate the association between ART and twinning rates and adverse perinatal outcomes, such as preterm births and birth defects. The findings revealed a notable decline in multiple pregnancies, attributed to the increasingly prevalent practice of transferring single embryos. This approach aligns with a focus on optimizing the health outcomes for both the mother and the developing fetus, reducing the risks and challenges often associated with multiple gestations, including preterm birth.
Other investigations, with a sociological perspective, has shown that little attention has been paid to the contribution of ART to desired family size, with the main objective remaining to reduce childlessness.
The interpretation of findings from studies on ART necessitates considering that the population udergoing ART differs from the general population in its demographic and

health profile. This population often presents underlying infertility issues and tends to be of advanced age.

The last topic discussed was the use of drugs in pregnancy. More and more women may need to be treated with medication during pregnancy due to chronic or new-onset clinical conditions, and monitoring of medication prescription patterns during pregnancy could be an invaluable tool for assessing compliance with recommended supplement and medication use in clinical practice. The first study in this field provided an overview of drugs' prescriptions in the last years.
The second study focused on use of antidepressants during the postpartum year found that mothers who experienced a preterm birth had an excess risk of antidepressant use, suggesting an association between preterm birth and maternal mental illness.

The impact of the COVID-19 pandemic was considered in studies where appropriate. The COVID-19 pandemic had a significant impact on maternity and maternal health care. Health systems have had to adapt to ensure the safety and well-being of mothers and their babies, and changes have been made to antenatal care and maternity services. This has enabled expectant mothers to receive essential health care without unnecessary exposure to health facilities.
As regard ART services, the restrictions due to the pandemic have had a marked effect on the number of ART-mediated births in Lombardy with an overall reduction of ART-mediated births in 2020 and 2021 in comparison with 2019. However, during 2022, an increase in ART-mediated births emerged.
In addition, pandemic seemed to impact on the use of antibiotics in the cohort analysed in the study regarding use of drugs during pregancy. This reduction in antibiotic use could reflect a reduction in infections, particularly respiratory and urogenital infections, due to the effectiveness of the containment and prevention measures against COVID-19 infection.

In conclusion, to promote and ensure healthy pregnancies, safe childbirth, and the wellbeing of mothers and couples, it is essential that attention and resources are focused on the ongoing and closely monitoring of reproductive and childbearing health. This allows for early detection and management of potential complications or risks, allowing for timely intervention in healthcare system and supporting an evidence-based decision making in the clinical practise.

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