Post-conceptional exposure to clomiphene citrate and congenital malformations: A cohort study

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Rebeccah NEHARD

Context

Clomiphene citrate : ovulation inductor

- 1/day for 5 days after having ruled out a pregnancy
- Can be increased to 2/day

Inadvertent post-conceptional exposures

- Confusion between menstruations and early pregnancy bleeds

Post-conceptional exposures raise concerns

- Teratogenic and embryolethal in animals (except non-human primates)
- Insufficient clinical literature
  - Limited sample size, n=250 (small case series and case reports)
  - Methodological limits

Objectives

Assess the existence of an association between maternal post-conceptional exposure to clomiphene citrate and:

Main objective

Major congenital malformations

Secondary objectives

Minor congenital malformations

Description of all congenital anomalies in exposed and unexposed groups

Methods

Study design and settings

- Retrospective exposed/unexposed cohort study
- *Centre de Référence sur les Agents Tératogènes (CRAT)*

Participants

Matching on year of contact

Exposed group	**Unexposed group**
Prospective records with known outcomes Gestational age at initial contact < 22 weeks after last menstrual period Delivery dates planned before February 2022	
CC at any time between 2 and 12 weeks after last menstrual period	No CC in the 3 months before conception or during pregnancy

Non-inclusion: exposure to teratogens, chromosomal abnormalities or genetic defects, unknown birth outcomes or incomplete records

Methods

Outcomes

- Main: major congenital malformations (EUROCAT)
- Secondary : minor congenital malformations (EUROCAT)
- Collegial assessment by the *CRAT* medical team, blinded to the exposures

Data source: *CRAT* medical database

- Maternal characteristics

  → Year of initial contact with the CRAT and gestational age at contact, maternal age, parity, history of congenital malformations and consanguinity
- Pregnancy outcome
- Diagnosis of any congenital malformations
- Neonatal characteristics for live-birth

  → Sex, birthweight, gestational age at birth, Apgar score…

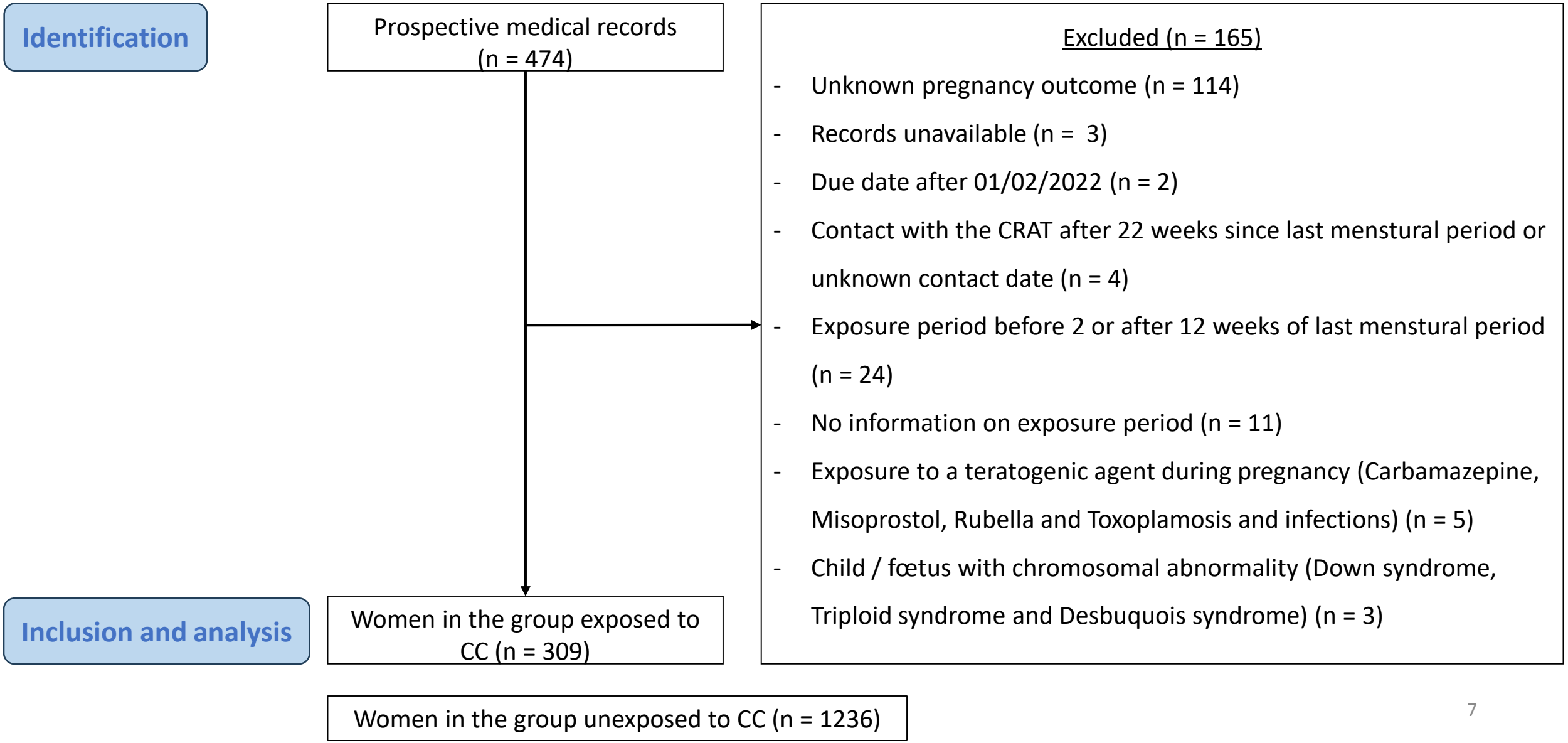
Methods

Statistical aspects

- Sample size:
  - Exposure group: 309 women (exhaustive eligible population)
  - major congenital malformations = 2.5% (general population),
    Relative Risk to be detected of 2.5 (substantial increase),
    α risk = 5%, power ≥ 80%
    → Unexposed group: 1236 women (1 exposed : 4 unexposed)

- Statistical analysis:
  - Missing values not replaced
  - P-value < 0.05
  - Population characteristics described and compared with chi-square / Fisher tests
  - Prevalence of malformations given as percentage and 95% confidence interval
  - Association between congenital malformations and post-conceptional exposures to CC : crude Relative Risk
  - Adjustment on maternal age

Results: flow diagram of the women exposed to clomiphene citrate



Results: population characteristics (1)

	Exposed group (N=309)		Unexposed group (N=1236)		
	n	%	n	%	p
Time of contact with the CRAT					
before 1990	85	27.5	328	26.5	0.97
1990-1999	134	43.4	536	43.4	
2000-2009	47	15.2	200	16.2	
since 2010	43	13.9	172	13.9	
Gestational age at time of contact*					
< 6	56	18.2	182	14.7	0.24
6-13	207	67.5	847	68.5	
14-21	44	14.3	207	16.8	
Maternal age (years)					
< 25	31	11.1	141	13.0	**0.0005**
25-29	111	39.8	320	29.5	
30-34	95	34.1	352	32.4	
35-39	36	12.9	198	18.2	
≥ 40	6	2.2	74	6.8	

* in weeks since last menstrual period

Results: population characteristics (2)

		Exposed group (N=309)		Unexposed group (N=1236)		
		n	%	n	%	p
Parity †						
	0	129	53.3	382	39.1	**< 0.0001**
	1	93	38.4	288	29.4	
	≥ 2	20	8.3	308	31.5	
Number of drug exposures #						
	0	212	68.6	563	45.6	**<0.0001**
	>1	97	31.4	673	54.4	
History of congenital malformations ‡						
	Yes	0	0.0	15	4.5	0.18

† not including ongoing pregnancies
# other than clomiphene citrate
‡ among women having given birth previously (exposed group: 113 and unexposed group: 332)

Results: association between CC exposure and pregnancy outcomes and malformations

	Exposed to CC (N=309)		Unexposed to CC (N=1236)				
	n	%	n	%	cRR	95% CI	P values
Pregnancy outcomes							
Live birth	257	83.2	1063	86.0			
Spontaneous miscarriage	30	9.7	69	5.6			
Elective termination of pregnancy	16	5.2	85	6.9			0.06
Medical abortion	3	1.0	10	0.8			
Stillbirth	1	0.3	7	0.6			
Ectopic pregnancy	2	0.6	2	0.2			
Major congenital anomalies							
Yes	4	1.3	19	1.5	0.80	0.28 - 2.32	1.00
Minor congenital anomalies							
Yes	9	2.9	10	0.8	3.60	1.48 - 8.78	**0.003**

→ No association for major congenital malformations

→ **Association** for **minor** congenital malformations

(Stable associations after adjusting for maternal age)

Results: Major congenital malformations in the exposed group

- 4 major congenital anomalies in total
- One major malformation per child and no clinical pattern

Organ system	Major anomalies	Exposure period (weeks after LMP)
Muscular	Trigger thumb	5.1 to 5.5
Urinary	Renal agenesis	3.5 to 4.2
Respiratory	Pulmonary adenomatosis	6.2 to 6.6
Digestive	Type III esophageal atresia	5.2 to 5.6

Results: Minor congenital malformations in the exposed group

- 9 minor congenital anomalies in total
- One minor malformation per child and no clinical pattern

Organ system	Minor congenital anomalies	Exposure period (weeks after LMP)
Skeletal	Left calcaneovalgus foot	6.0 to 6.4
Muscular	Hip subluxation	6.0 to 6.5
Urinary	Pyelocalyceal dilation	3.4 to 4.3
	Bilateral hypotonia of the renal pelvis	4.0 to 4.5
Cardiovascular	Patent ductus arteriosus	7.4 to 8.3
Reproductive	Cryptorchidism	5.1 to 5.3
	Mucosal-vaginal prolapse	1.5 to 2.2
	Testicular ectopia	5.0 to 5.4
Digestive	Pyloric stenosis	3.0 to 3.4

Discussion: key results

- Largest cohort of prospectively followed women (n = 309)

- **No association** between post-conceptional CC exposure and **major** congenital malformations
    - Prevalence in exposed group: 1.3%, 95%CI [0.4; 3.3]
    - Prevalence in unexposed group: 1.5%, 95%CI [0.9; 2.4]
    - **cRR = 0.80** (95%CI [0.28; 2.32])

- **Association** between post-conceptional CC exposure and **minor** congenital malformations
    - Prevalence in exposed group: 2.9%, 95%CI [1.3; 5.5]
    - Prevalence in unexposed group: 0.8%, 95%CI [0.4; 1.5]
    - **cRR = 3.60** (95%CI [1.48; 8.78])
    - **No homogenous clinical pattern**

Discussion: minor congenital malformations

Significant association between post-conceptional CC exposure and minor congenital malformations

- **No specific pattern of congenital malformation in the offspring**
- **Association linked to chance?**
- **Biased association?**
    - **Notification bias?**
      Better description of minor congenital malformations in exposed women
    - **Confusion bias?**
      No adjustment on confounding risk factors of congenital malformations

Discussion: strengths and weaknesses

Strengths	Weaknesses
• Data collected **prospectively** from **healthcare professionals** in a single centre • **Comparative study** (not only descriptive) • Major / minor congenital malformation categorisation ascertained by the *CRAT's* medical team, **blinded to the exposure**	• Important amount of **missing data:** impossible to adjust for potential **confounding factors** • Sample size too small to study the association between exposure and occurrence of **specific congenital malformations**

Discussion: previous clinical studies

- **Preconceptional exposure studies:**
  - Increased risks of neural tube defects* and hypospadias**?
    → Small study samples
    → Results rarely adjusted for confounding factors
    → Periconceptional use (meta-analysis Auffret *et al.* 2019): no significant increase in neural tube defects

- **Postconceptional exposure studies:**
  - About 250 cases reported
    → No patterns of congenital malformations

* Wu *et al*. 2006
** Meijer *et al.,* 2006, Lind *et al.,* 2013, Sørenson *et al*., 2015

Conclusion

- **No association between CC exposure and major congenital malformations**
- **Significant increase of the risk of minor congenital malformations**
  - Interpret with caution
- Further studies needed
  - Other (collaborative) data

    → Inclusion of more women

    → Adjusting on more confounding factors

    → Different unexposed groups comparable in terms infertility and related clinical context and outcomes

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CRAT

Thank you for your attention!

Special thanks to:

The CRAT team

Healthcare profesionals having contacted the centre for patients included in our cohort

Supplementary slides

Table: Major congenital malformations in exposed and unexposed groups to clomiphene citrate (CC)

Organ system	Major anomalies	Exposed to CC	Unexposed to CC
Skeletal	Polydactyly		1
	Hexadactyly		1
	Upper limb anomaly		1
	Equinovarus foot		1
	Symbrachydactyly (missing 4 fingers) + clubfoot		1
	Bilateral equinovarus clubfoot		1
	Craniosynostosis		1
Nervous	Spina bifida and ventricular dilation		1
	Microcephaly + incomplete brain development		1
Muscular	Trigger thumb	1	
Urinary	Duplicated kidney (regression at 6 months)		1
	Renal agenesis	1	
	Suspected bilateral polycystic kidney disease		1
Cardiovascular	Atrial septal defect		1
	Large non-restrictive peri-membranous Ventricular Septal Defect and mild pulmonary stenosis		1
	Patent ductus arteriosus		1
Respiratory	Pulmonary adenomatosis	1	
Reproductive	Hypospadias		2
Digestive	Gastroschisis - Digestive atresia with perforation		1
	Type III esophageal atresia	1	
	Omphalocele		1
Other	VACTERL syndrome, cord atresia with thrombosis		1
Total		4	19

Table: Minor congenital malformations in exposed and unexposed groups to clomiphene citrate (CC)

Organ system	Minor congenital anomalies	Exposed to CC	Unexposed to CC
Skeletal	Varus of the forefoot		1
	Left calcaneovalgus foot	1	
	Bilateral varus of the midfoot		1
Muscular	Hip subluxation	1	
	Hoffman syndrome and naevus		1
Urinary	Pyelocalyceal dilation	1	1
	Bilateral hypotonia of the renal pelvis	1	
Cardiovascular	Patent ductus arteriosus	1	
	Heart murmur		1
Reproductive	Cryptorchidism	1	
	Mucosal-vaginal prolapse	1	
	Testicular ectopia	1	
Digestive	Pyloric stenosis	1	3
Other	Short lingual frenulum		1
	Tongue-tie (ankyloglossia)		1
Total		9	10

Table: Epidemiological case-series of post-conception exposures to clomiphene citrate

Population size	Pregnancy outcome	References
n = 158	7 congenital malformations	Merrell National Laboratories, 1976 [4]
n = 25	5 pregnancy terminations 3 miscarriages 16 live births including 1 congenital malformation (fibular agenesis)	Carlier et al., 1996 [2]
n = 35*	2 spontaneous abortions (at 9 and 21 weeks of gestation) 2 births with congenital malformations (hydrocele: exposure at 3 weeks, diaphragmatic hernia: exposure at 5 weeks) 31 live births with no malformation	De Vries et al., 2014 [3]

*exposures at any time between 2 and 14 gestational weeks

Data collection

1rst contact with CRAT
(prospective medical records)

Data source:
Clinical database of CRAT

E+ clomiphene citrate

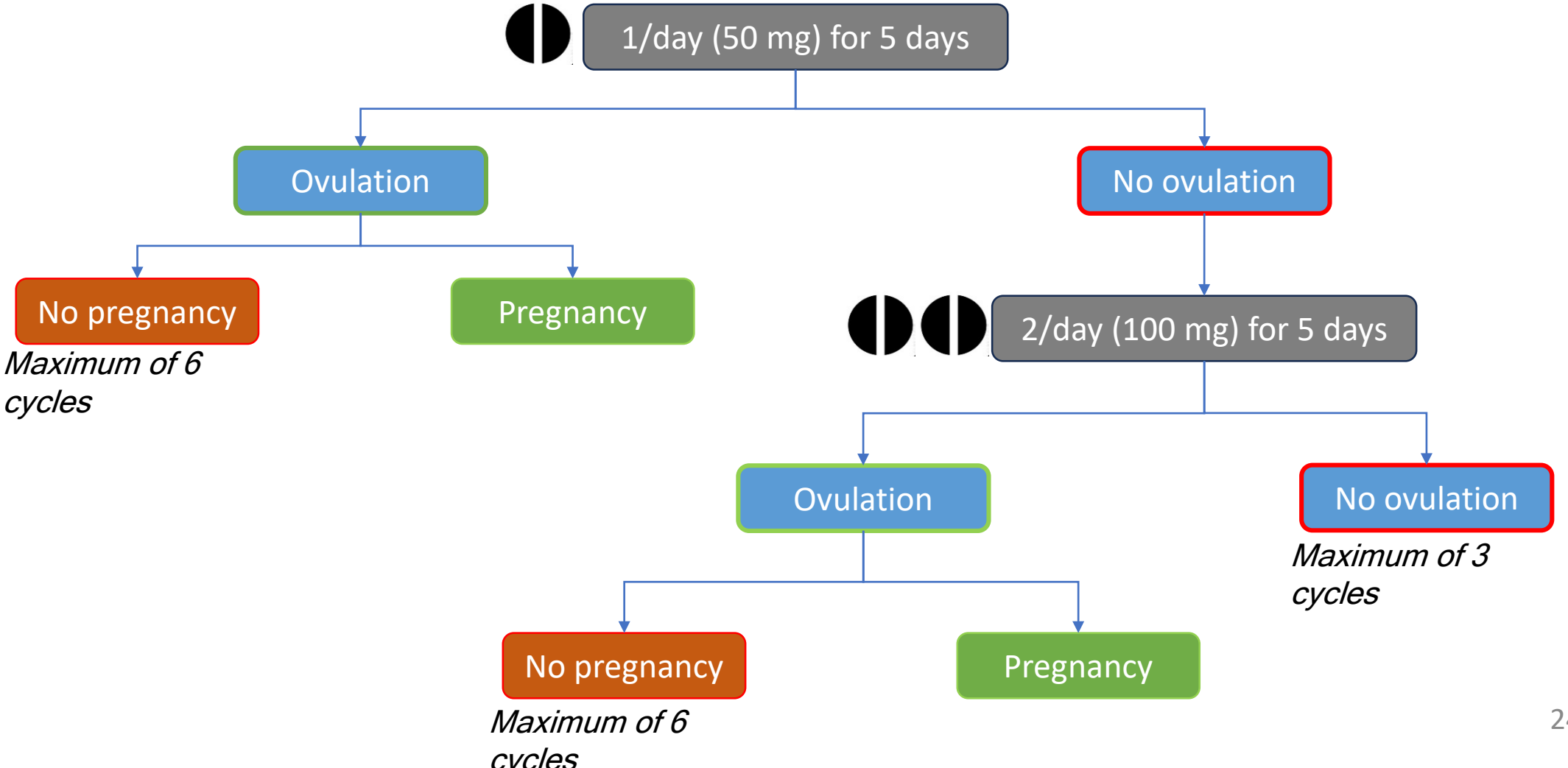
E- clomiphene citrate

« Morphological » echography of the 2nd trimester

2nd contact with the CRAT : data collection on birth outcome

Clomiphene citrate (CC) and pregnancy

- Prescribed as an ovulation inductor (2 to 5 days after the beginning of menstruation)
- **After having ruled out a pregnancy**



Regulatory aspects – data collection

- Medical database CRAT authorized by CNIL in 1989 (number 2021092110154)
- **Current study:**
  - Research not involving the Human person
  - Following reference methodology MR-004 *CNIL* (number 20220516167745)

https://www.cnil.fr/fr/declaration/mr-004-recherches-nimpliquant-pas-la-personne-humaine-etudes-et-evaluations-dans-le

e-Table 1: Preclinical studies of the effects of CC on embryo-fetal and post-natal development:

Species	Doses	Exposure dates	Observations	References
Table 1a: In utero exposures to CC (rodents)				
Mice	1, 2, 4 and 6 mg/kg (PO)	GD 8	Morphological, morphometric and histological anomalies	Ara and Asmatullah, 2011 [22]
Rats	2 mg/kg	GD 5-12	Cellular anomalies of the reproductive organs	McCormack and Clark, 1979 [23]
	Single administrations of 2, 10, 50 or 200 mg/kg (SC)	GD 6, 8, 10, 12 and 14	Hydramnios and congenital cataracts	Eneroth et al., 1970 [24]
Table 1b: In utero exposures to CC (non-rodents)				
Lagomorph - rabbit	7.5 mg/kg	GD 1	Gastroschisis, cranioschisis, ablepharia, short limbs, cleft palates	Morris, 1970 [25]
	20 mg/kg	GD 2	Hydrocephaly	
Non Human Primate – Macaca mulatta	1.5 – 4.5 mg/kg/day (PO) (similar to clinical doses)	1.5 mg/kg at GD 20-22 2.0 mg/kg at GD 23-25 3.0 mg/kg at GD 16-36 4.5 mg/kg at GD 23-25 or at GD 24	No fetal anomalies	Courtney and Valerio, 1968 [26]
	0.75 – 1.5 mg/kg/day (IM)	0.75 mg/kg at GD 17-29 1.5 mg/kg at GD 23-25		
Table 1c: Post-natal exposures to CC (rodents)				
Mice	20 mg SC pellets	nr	Hyperplastic epithelial of the vagina (similar to diethylstilbestrol)	Cunha et al., 1987 [19]
	5 µg/day	PND 1-5	Histological urogenital anomalies	Gorwill et al., 1982 [20]
Rats	10 to 500 µg (SC)	PND 1	Anomalies of the female reproductive organs (similar to diethylstilbestrol)	Clark et al., 1977 [21]

Legend: GD: gestational day; PO: per os; SC: subcutaneous; IM: intramuscular; nr: not reported; PND: post-natal day

Prévalence de malformations congénitales majeures

1,3% dans le groupe de femmes exposées au CC
1,6% dans le groupe de femmes non-exposées au CC

→ Sous estimation par rapport à fréquence en population générale : de **2,6%** (selon EUROCAT).

- Exclusion des tératogènes avérés chez l'Homme ?
  (mais la part de MCM liées à une exposition médicamenteuse en période d'organogenèse est estimée à seulement 5%)

- Profil particulier des patientes pour lesquelles un TIS est contacté ?
  (non représentatives de la population générale en termes de connaissances et d'informations relatives à la santé périnatale ?)

- Après exclusion des anomalies génétiques, l'incidence de MCM de **1,98%**

- Différences précédemment constatées dans les travaux des TIS (Weber Schoendorfer et al., 2015; Vauzelle et al., 2013; Guilbaud et al., 2019; Hatakeyama et al., 2022).