Superfecundation – from ancient to modern times

Review article

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Summary
Superfecundation is an extremely rare phenomenon. It occurs when the second ovum (released in the same menstrual cycle) is fertilized by sperm cells from separate sexual intercourse. Superfecundation can occur only when a woman has had sexual acts within a short period of time from one another.
The article describes the phenomenon of superfecundation as an example of multiple pregnancy in which people have been interested since antiquity. The paper discusses factors favoring the occurrence of superfecundation and presents the current state of scientific knowledge on the topic with case reports.
Key words: superfecundation; multiple pregnancy; polyovulation; mythology; *in vitro* fertilization

INTRODUCTION

A pregnancy during which more than one embryo develops in the uterus is called a multiple pregnancy [1]. Such pregnancies are usually encountered in women of the black race. They account for 2–4.5% of all pregnancies in this ethnic group. In white women, such pregnancies are moderately frequent – they constitute up to 1.4% of all pregnancies. Multiple pregnancies are the rarest in Asian women – merely 0.5% of pregnancies. It has been agreed that the frequency of natural multiple pregnancies decreases geometrically with an increase in the number of fetuses developing in the uterus. This dependency is called Hellin's law and was named after its creator (1895) [2]. It estimates the probability of multiple pregnancies using the formula presented in Fig. 1.

This stochastic model is based on the assumption that multiple pregnancies are conditioned only by additional ovulations (polyzygotic pregnancies) and divisions of an already existing ovum (monozygotic pregnancies). However, due to the fact that fertilization and the rapidly following cell divisions in the developing embryo are subject to various interfering factors, the Hellin's law is merely an approximation of the actual frequency with which the phenomenon of multiple pregnancy occurs [3]. In 1993, Fellman and Eriksson proved in a mathematically unambiguous way that this dependency cannot be treated as a general principle but merely as an approximation [4]. It is estimated that the frequency of twin dizygotic pregnancy in Poland is approximately 1% [5].

Figure 2 presents the estimated frequency with which twin, triplet, quadruplet and quintuplet pregnancies occur according to the Hellin's law.

The main factors (Fig. 3) that increase the probability of multiple pregnancy mainly include: mother's race, age, height (above 170 cm), body weight (BMI over 30 kg/m^2) and obstetric history (number of births, history of a multiple pregnancy, using ovulation-stimulating agents and oral contraceptives) [5–8]. Moreover, socioeconomic factors also play a role, such as: the mother's marital status [9,10], affluence [11] and her habits: cigarette smoking [12–14], alcohol use [6,14] or drinking caffeine-rich drinks [13,14]. A relatively "new" factor that affects the occurrence of multiple, mainly twin pregnancies is increased popularity of assisted reproductive technology, such as *in vitro* fertilization, intrauterine insemination or ovulation induction [7,15,16].

Numerous studies have confirmed that twin pregnancies are significantly more common in women aged 35–39 compared with both younger and older women [6,7]. This is probably associated with gonadotropin secretion which increases with age. Also, this age marks the greatest secretion of gonadotropic hormones, the consequence of which is the maximal stimulation of ovarian follicles. This also explains lower fertility in older women when ovarian function gets weaker. It is speculated whether genetic and environmental factors as well as more frequent spontaneous abortions in older women can affect decreased birth rates in women above the age of 40 [6]. Furthermore, it has been proven that multiparity (more than 3 childbirths) can increase the probability of multiple pregnancy [5,8]. Also, twin pregnancies are more common in women who are twins themselves [17].

POLYOVULATION AND SUPERFECUNDATION

Superfecundation (*superfecundatio*) is an extremely rare phenomenon and one of complications of multiple pregnancy. It occurs when the second egg cell (released during the same menstrual cycle) is additionally fertil-

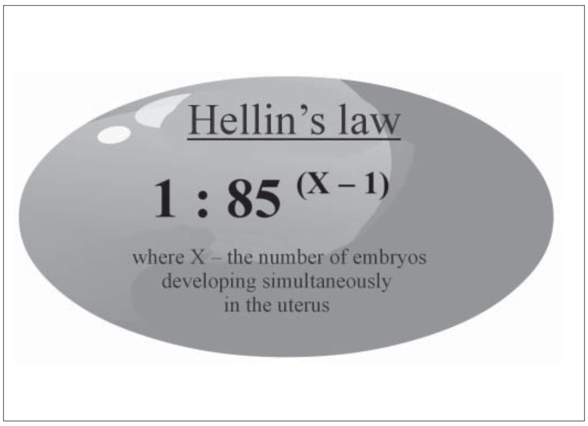


Fig. 1. Hellin's law – formula

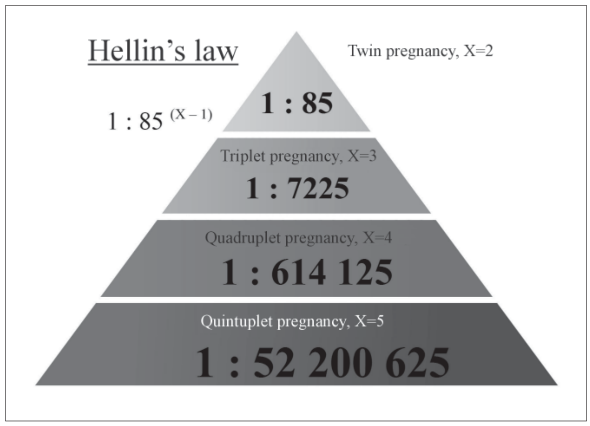
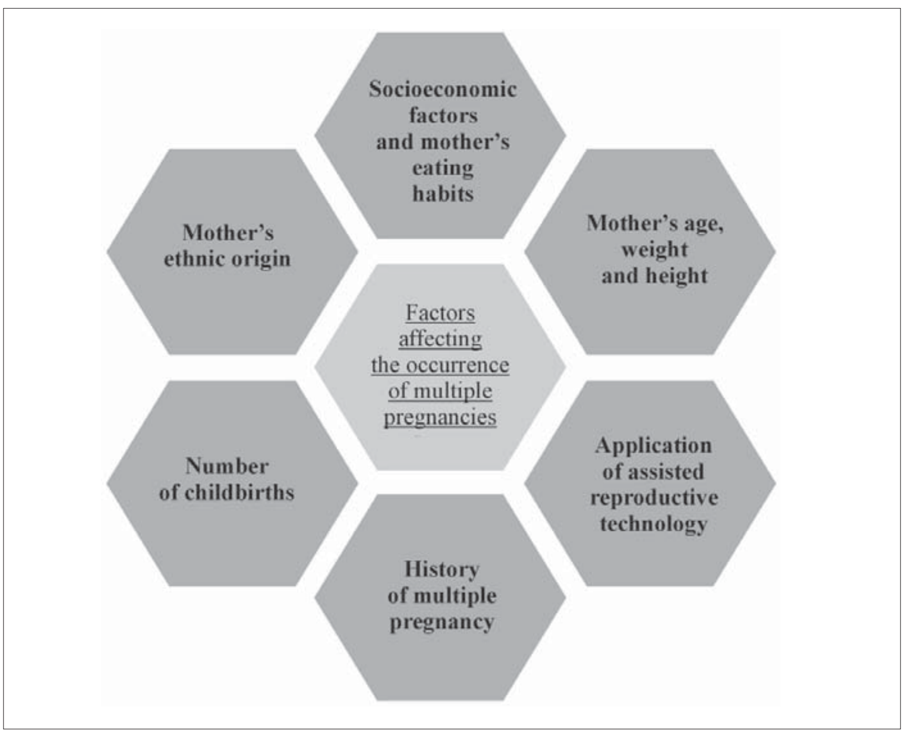


Fig. 2. Frequency of multiple pregnancies

Fig. 3. Major factors affecting the occurrence of multiple pregnancies



ized by sperm cells from separate sexual intercourse which takes place within a short period of time from the first intercourse. These sperm cells can belong to the same partner or to different men. The second situation is referred to as *heteropaternal superfecundation* [5]. It is assumed that the second fertilization occurs between the third and fourth day from the day of the first insemination. It seems likely, however, that the period of time between two fertilizations can be longer, even to 14 days [5,18].

A condition necessary for superfecundation to occur and a dizygotic pregnancy to develop is the occurrence of polyovulation. After fertilization, many changes take place in the woman's body. They are determined by the production of hormonally active compounds that prevent another ovulation from occurring. The main hormone responsible for this inhibition is progesterone. As an ovarian follicle ruptures and transforms into the corpus luteum, the ovary starts producing and secreting progesterone. An additional source of this hormone is the placenta that develops in the uterus. An increase in progesterone levels results in the activation of the hypothalamic–pituitary–gonadal axis and the inhibition of pituitary hormones (follicle-stimulating hormone – FSH and luteinizing hormone –LH), which prevents another ovulation. Spontaneous sequential occurrence of multiple ovulations during one menstrual cycle is an extremely rare phenomenon [19,20]. However, it can result from ovulation induction with the use of human chorionic gonadotropin [20–23], clomifene citrate (estrogen antagonist) [24–27] or gonadoliberin [28–30].

Moreover, following fertilization, an increase in progesterone levels causes changes in the structure (thickening) and quality (increased mucin level – increased viscosity) of the cervical mucous thereby preventing the passage of sperm cells through the cervical canal and their further penetration through the genital tract to the site of fertilization – the tubal ampullae [31–33]. Cervical mucous is also a site where sperm cells are stored. It protects them from phagocytosis and unfavorable conditions of the vagina. Moreover, it is a source of energy substances and facilitates the penetration of sperm cells to the uterus during ovulation [34]. Therefore, it seems likely that polyovulation enables sperm cell penetration to the uterine cavity. This can result in additional fertilization – superfecundation.

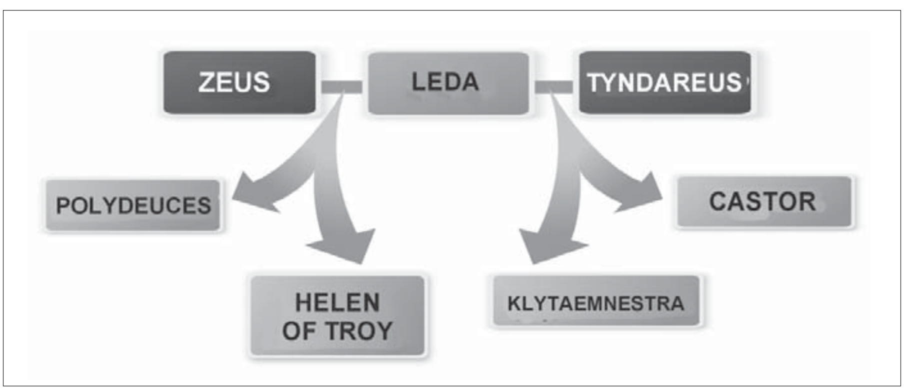
SUPERFECUNDATION – ANTIQUITY

The phenomenon of superfecundation has been known for over two thousand years. The phenomenon of having twins or more children at once was tried to be explained even in antiquity. The issue of a multiple pregnancy can be found both in the literature and art of each of European cultural periods. Greek and Roman mythology present beliefs concerning the origin and delivery of twins. Ancient people believed that a woman who was a mere mortal and had sexual intercourse with a man–god and then with a mortal on the same day or night, can deliver several children: a half-god fathered by a man of godly origin, and a mortal – a child fathered by a man who was not endowed with superhuman, divine powers. A mythical example of superfecundation can be the origin of Helen of Troy, her sister Klytaemnestra and brothers Castor and Polydeuces. Their mother was Leda – an Aetolian princess and the queen of Sparta. As one of the versions of this myth say, Leda had sexual intercourse with Zeus, who had seduced her in the guise of a swan, and her husband – Tyndareus during the same night. Leda gave birth to twins to both men. Zeus was the father of Helen and Polydeuces, and Tyndareus fathered Castor and Klytaemnestra [19,20,35–40]. Figure 4 presents a part of Leda's descendants.

The motif of Leda and the swan was popular among artists in the subsequent ages. It can be found mainly in works of: Michelangelo, Leonardo da Vinci, Salvador Dali, François Boucher, Peter Paul Rubens, Gustav Klimt or Louis de Silvestre [20,36]. Moreover, one of planetoids, discovered in Paris by Jean Chacornac in 1856, was named after mythical Leda [41].

Another example of heteropaternal superfecundation in antique literature is the genealogy of a half-god who is undoubtedly the most famous hero in European culture and whose numerous adventures are described in the mythology. It is Heracles. Officially, he was the son of Alcmene and Amphitryon – the king of Tiryns.

Fig. 4. Descendants of Leda, the queen of Sparta



However, god Zeus was his real father. The mythology says that he seduced Alcmene in the guise of her husband and that is how Heracles was fathered. However, Amphitryon returned early from his military campaign the same night. As a result of another intercourse, Alcmene conceived the second child – the rightful heir and a son of a mortal man, named Iphicles [19,20,35–40]. Figure 5 presents a part of Alcmene's descendants.

The differences between these two boys were presented on small Attic clay jars (*stamnos).* Heracles was presented as a fair-haired young man whereas his brother had dark hair. The most prominent difference, however, was strength and courage of Heracles while Iphicles lacked these qualities. Young Heracles boldly fights the snake depicted on the vase whilst horrified Iphicles hides in his mother's womb and is protected by her hands [36,38]. These examples prove that even in antiquity and in the subsequent ages people tried to explain the nature of multiple pregnancy in a non-scientific way. Based on the observation of twins and differences in their appearance and behavior, the fact of having more than one child at once was frequently attributed to supernatural divine powers. The explanation of this phenomenon was searched for in myths, legends and folklore.

SUPERFECUNDATION AT THE AGE OF SCIENCE

The earliest reports of superfecundation come from 1714. They concern two children of different skin colors born of one woman. Most of the first cases of superfecundation reported by Gould and Pyle refer to black women who admitted to have had intercourse with their husbands (of the black race) and a white man within a short period of time. These women were frequently servants, and the intercourse with their superiors was frequently forced [19,20,42]. It seems then that having children of different races is sufficient evidence of heteropaternal superfecundation.

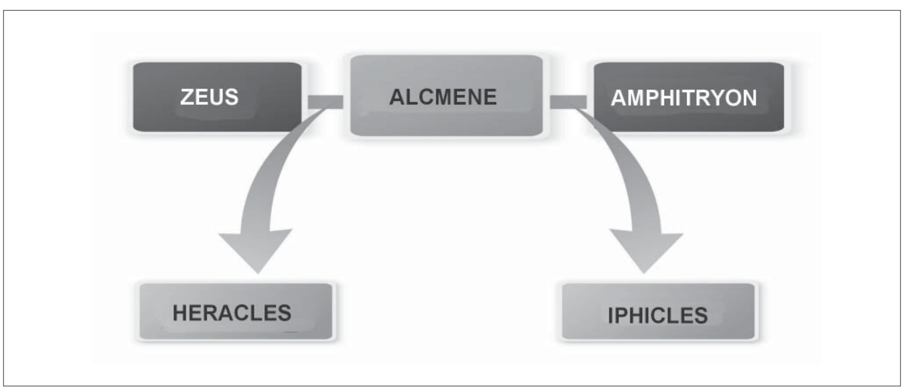
In 2010, however, Claas et al. reported a case of a 22-year-old woman of Negroid origin who gave birth to dichorial diamniotic two daughters of a different phenotypic appearance. One of the twins had light brown skin, black curly hair and brown eyes, whereas the second child had white skin, red-blond curly hair and blue eyes. It was initially suspected that the twins were conceived by superfecundation. Genetic studies, however, did not confirm this hypothesis. It was proven that the second girl suffered from oculocutaneous albinism. In such cases, the differential diagnosis should include superfecundation and congenital albinism [43].

The frequency of occurrence of heteropaternal twins in humans was estimated in 1992 by a team of American scientists led by Wenk. According to their analysis of a large data base consisting of 39,000 cases of doubtful paternity, superfecundation was scientifically confirmed in only three cases. The frequency of this phenomenon was then estimated at 1 per 13,000 cases. It was also determined that heteropaternal twins can constitute even 2.4% of all dizygotic pregnancies [44]. According to James, every twelfth pair of dizygotic twins is a consequence of monopaternal superfecundation. The same author also suspects that heteropaternal origin concerns even one per 400 pairs of twins among marriages of white Americans with twins [45].

Based on genetic tests, Bellis and Baker have proven that heteropaternal children can constitute even 5% of dizygotic twins [46]. The frequency with which this phenomenon occurs apparently depends on the intensity of sexual life of both men and women in a given population. That is why, these numbers are probably underestimated. In certain populations, this phenomenon can be much more common, e.g. among women engaging in prostitution [19,20,35,47].

The literature contains only several dozen cases of twins proven scientifically to have originated from superfecundation [1]. Many authors particularly emphasize the differences between birth weights of twins. This is usually explained with a different gestational age of children, which, in turn, is a consequence of time that passed from the fertilization of the first egg cell to the second insemination [48,49]. Girela et al. report that the difference in birth weight of twins was 650 g and the "younger" child had problems with the adaptation to living beyond the maternal uterus (hyperbilirubinemia, anemia and risk of septic shock). The child was transferred to the intensive care unit and discharged in a good

Fig. 5. Alcmene's descendants



overall condition after 6 weeks [47]. Bertrams and Preuss report the difference in birth weight of 450 g [50]. Amsalem et al. described twins whose birth weights differed by 220 g [51], and Peigne et al. presented discrepancies reaching even 460 g [52]. Table 1 presents examples of birth weight differences between children conceived via superfecundation.

For years, various laboratory methods have been used to confirm or exclude paternity. Not in all cases, however, could paternity be unequivocally established since the biological material from the second "potential" father could not always be obtained. Sometimes, superfecundation and its consequences could be only suspected mainly due to testimonies of mothers who admitted to having had multiple sexual partners [48].

Verma et al. used cytogenetics to document the heteropaternal origin of twins [49]. Bertrams and Preuss [50], Terasaki et al. [53], Spielmann [54], Lu et al. [55], Lebeau-Le Guiner et al. [56] and Girela et al. [47] used classical serology – they performed analyses of blood type markers: hemagglutination assay for histocompatibility complex for ABO, Rh, MNS, Duffy, Kidd, Kell-Cellano and Lutheran systems. Moreover, authors also used isoelectric focusing of erythrocyte enzymes (phosphoglucomutase 1 and acidic phosphatase) and serum proteins (transferrin and alpha-1 antitrypsin). However, these methods are not perfect [57]. In 2003, Lebeau-Le Guiner et al. demonstrated that standard serological testing conducted to confirm/exclude paternity in heteropaternal twins were insufficient. Only by DNA analysis was the tested man excluded as being the father of one twin and confirmed to be the father of the second child [56].

Within the past 25 years, various DNA sequence analyses, which have been developing intensively, have played an enormous role in human genetics, molecular diagnostics of diseases and forensic medicine. These analyses are currently classified into 5 main types:

1. restriction fragment length polymorphism (RFLP);
2. short tandem repeats, also called microsatellite (STR);
3. variable number of tandem repeats (VNTR);
4. single nucleotide polymorphism (SNP);
5. copy number variation (CNV) [57].

These methods are also applied in proving the heteropaternal origin of twins. As early as in 1997, Girela et al. used the RFLP test and confirmed that the tested man was the father of one of twins; the probability reached 99.9999998% after examining 25 genetic markers [47]. In 2001, Geada et al. applied short tandem repeats (STR) and restriction fragment length polymorphism (RFLP) and determined 37 genetic markers in putative fathers and the mother. The test unambiguously showed (with the probability of 99.999991%) that man 1 was the father of one of the children. As for the other child, as many as 10 loci tested showed incompatibility. Moreover, it was demonstrated that man 2 was the father (with the probability of 99.99999992%) of the second child whereas his putative paternity with respect to the first child was ruled out based on the incompatibility of the genetic material in 14 loci. In this case, the paternity of the second man is certain despite genetic incompatibilities between him and the child in two genetic markers tested. It turned out that Geada et al. were the first to discover and describe an STR mutation concerning gene ACTBP2 [58]. The study conducted by Lebeau-Le Guiner et al., using STR DNA analysis, definitively confirmed paternity with the probability of 99.9995% [56]. In 2008, Hansen and Simonsen unequivocally confirmed another case of superfecundation in Danish twins. Based on an STR analysis of 15 autosomal genetic markers and 11 markers located on chromosome Y, it was proven that the children had different fathers [59].

In the recent years, an increase in the usage of assisted reproductive technology in infertility treatment centers resulted in reports concerning cases of superfecundation in women who have undergone *in vitro* fertilization. The problem of absolute sterility or partial fertility limitation concerns approximately 1.2 million pairs in Poland. The Division of Fertility and Sterility of the Polish Gynecologic Society report that in 2011 there were 15,340 cycles treated with assisted reproductive technology, including 10,011 *in vitro* fertilization procedures. These numbers are projected to increase each year. This is determined by pro-family actions undertaken as well as increasing social needs and expectations [60]. In 2001, Amsalem et al. reported the first genetically confirmed case of monopaternal superfecundation in a 25-year-old woman who had undergone a procedure of *in vitro* fertilization. This procedure was conducted due to secondary unexplained infertility diagnosed in the patient (hysteroscopy, hysterosalpingography and semen evaluation were normal). The patient underwent a procedure, during which two

Tab. 1. Birth weight differences in children conceived via superfecundation (based on [47,50–52])

Authors	Year of publication	Birth weight of the 1st twin	Birth weight of the 2nd twin	Birth weight difference
Bertrams and Preuss	1980	3350g	2900g	450g
Girela et al.	1997	2100g	1750g	650g
Amsalem et al.	2001	2390g	2170g	220g
Peigne et al.	2011	1240g	780g	460g

embryos were transferred to the uterus. 20 days after the procedure, a transvaginal US scan showed two gestational sacs. A week later, however, five sacs were visualized, each with its own heartbeat. The patient and her partner confirmed that they had had intercourse within a short period of time from the embryo transfer. In week 12 of pregnancy, it was decided to conduct selective reduction of three embryos by injecting KCl solution and inducing asystole. The further development of the twin pregnancy was not complicated. The woman gave birth to two male children in the 37th week of gestation via a cesarean section. The genetic analysis of three reduced fetuses and two living twins demonstrated unambiguously that the three unplanned embryos resulted from fertilization (regular intercourse) of oocytes originating from an additional ovulation which took place after the procedure of intrauterine insemination [51]. 10 years later, Peigne et al. reported a similar case in a 31-year-old woman who had been treated for infertility together with her husband for 5 years. The diagnostic hysterosalpingography was normal and menstrual disorders were attributed to changes in the patient's hormonal imbalances, which could suggest polycystic ovary syndrome. Semen evaluation showed asthenozoospermia. The patient underwent 3 ineffective procedures of intrauterine insemination. Finally, *in vitro* fertilization was conducted. As in the example mentioned above, a transvaginal US scan showed the presence of five live embryos, three of which were selectively reduced. The woman gave birth to twins of different sexes in the 31st week of gestation via a cesarean section. The analysis of parental DNA as well as genetic material obtained from the live twins and three embryos confirmed that the additional embryos were fertilized via superfecundation. The parents admitted to having had sexual intercourse on the day after the insemination procedure [52].

CONCLUSION

The literature mentions few cases of superfecundation in humans. People have been interested in multiple pregnancies since antiquity. In ancient times, such pregnancies were frequently understood as mystical or divine phenomena. The frequency of superfecundation in the general population is low. It seems probable that it can be more frequent in certain social groups. This hypothesis, however, would require verification in additional studies. With the development of laboratory techniques and the possibility of conducting genetic tests, unequivocal paternity determination in disputes concerning heteropaternal twins has become possible.

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