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The role of embryopathology in the study of first trimester spontaneous miscarriage

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Abstract

The study of first trimester spontaneous miscarriage is the object of increasing attention not only for clinicians but also for patients and their family. Embryopathology concerns the study of pathologic conditions and malformations like early development failure, development alterations and true malformative lesions. It is well known that the most severe genetic abnormalities emerge as an interruption in embryogenesis and that the most complex developmental abnormalities caused by external factors - like infections, toxic and environmental agents - have their onset in the early stages of embryo formation and are often also the cause of pregnancy loss. In order to improve the monitoring of at risk couples we have expanded the study of basic research with those of research applied to human pathology. The role of embryopathology is fundamental to understand the timing at which the embryo development failure and its subsequent death actually occur and the main defect in embryo formation underlying development abnormalities. From 1994 to 2010 we examined 256 early spontaneous abortions in which an intact gestational sac was present. The introduction of acrylic resin embedding technique in 1995 added many advantages such as the possibility of obtaining semi-thin and uniformly thick sections with better histological and cytostructural details, assuring the almost total absence of coartation, and the preservation of antigenic properties. This technique therefore permits improvement in morphological observation in order to reinterpret the classification of spontaneous aborted embryo. This enables a better definition of the real stage and time when development was interrupted. That is the basis to understand the role of external factors as a cause of pregnancy loss.

Introduction

There are different classifications employed to describe the embryo in first trimester spontaneous abortion. Most of them are outdated and are based on macroscopic examination only.

The Fujkura classification (1966) [1] is based on the integrity of the gestational sac, the presence or absence of the embryo and the classification of the latter as nodular, amorphous or cylindrical. The classification introduced by Poland (1981) [2] divides deformed embryos into nodular (length from 1 to 4 mm), cylindrical (up to 10 mm) or stunted (over 10 mm). These classifications require solely morphological studies based on stereomicroscope dissection. Nodular embryos representing early development failure, are the main problem. The identification of more anatomical details is relevant in order to establish the embryo's real stage of growth and organization.

The analysis of embryos by stereomicroscope dissection and semi-thin sections embedded in acrylic resin [3], permits the acquisition of better morphological detail even in case of autolysed and poorly preserved samples. Stereomicroscope dissection is used to accurately define the existence of an empty chamber, to reveal appendages, like the yolk sac or the body stalk. It also identifies even a fraction of umbilical cord which is often the only visible part in case of autolysis [4]. Specimens obtained by routine paraffin embedding do not allow accurate morphologic observations. Conversely, plastic embedding is free from distortion or artefacts due to paraffin embedding and maintains well preserved cellular connections without shrinkage.

The classification of embryos made by the Carnegie Institute of Embryology [5] with the study of the presence or absence of various anatomical structures, helps to identify the precise point at which arrest of development occurred, often much earlier than the time of expulsion [6]. Since 1995 we use this technique for our cases in order to improve the determination of the chronology of events leading to pregnancy loss [7].

Materials and methods

From 1994 to 2010 we examined 6600 cases of first trimester spontaneous miscarriage in our Centre of Gynaecopathology and Embryopathology.

In this series we identified 256 cases in which a gestational sac was present and which satisfied the following clinical criteria.
Clinical criteria for enrolment.
Only cases in which these criteria were fulfilled were enrolled:
1) 'Truly spontaneous'miscarriage: (no history of interruption of pregnancy with drugs that induce abortion, eg. Cytotec, ergotamine, methotrexate)
2) duration of amenorrhea between 6 G.W. (gestational weeks) (5w0d) and 13 G.W. (12w6d = 90D), referred by clinicians or otherwise calculated based on the date of last menstruation.
Cases of assisted reproduction (In vitro Fertilization-IVF, gamete intra-fallopian transfer-GIFT) were also considered and the main criteria were:
1) pregnancy achieved by assisted reproductive procedures (assisted fertilization-FA), when possible with details on the type of method used [8];
2) spontaneous pregnancy, but with a history of FA procedures performed in the past [8];
3) pregnancy obtained after other assisted reproduction procedures (primarily hormonal stimulation) [8].
Then they were studied as per protocol.
The protocol describes the operating procedure [9] for the embryo-pathological diagnosis, which includes:
A) observation and careful dissection of the gestational sacs that are positioned along the longitudinal axis on the dissecting microscope (M651-Leica) (Fig. 1);

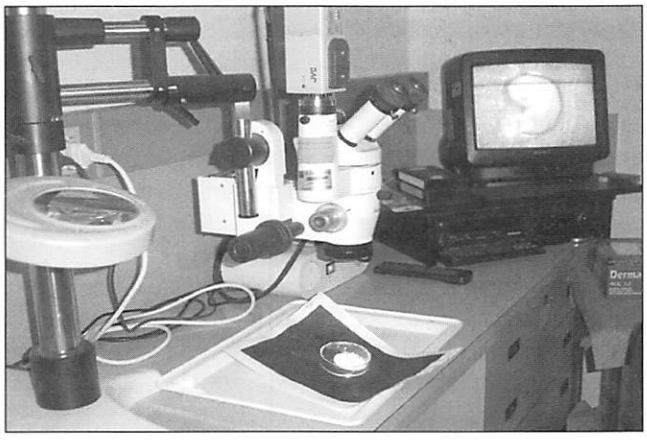
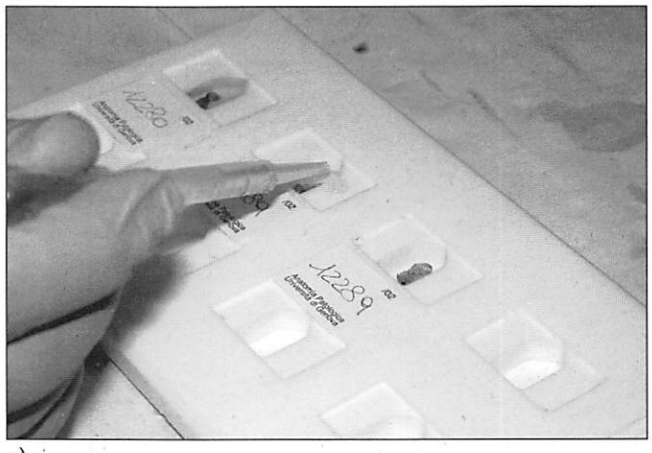
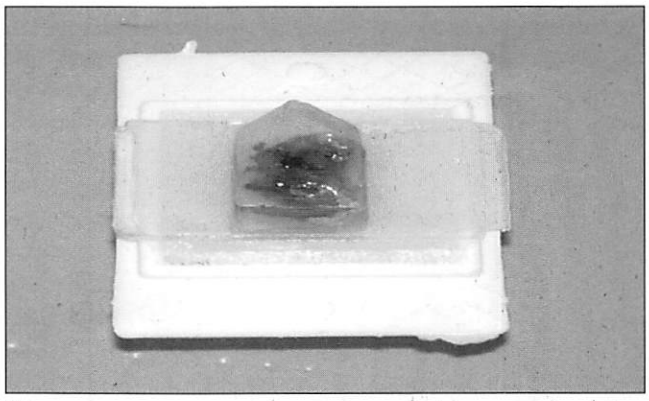


Figure 1. The dissecting stereomicroscope (M651-Leica).

B) embedding in acrylic resin.
The resin employed in this method is Technovit 8100 (Kulzer, Germany), with cold cure. It is a combination between hydrosoluble resin (HEMA) and plasticizing resin (hydroxy-ester).
The steps of this method consist in:
FIXATION: alcohol and ether for 24 hours.
DEHYDRATION: absolute alcohol.
IMPREGNATION: vacuum for 72 hours in solution A and the hardener (catalyst benzoyl-hydrate).
RESIN EMBEDDING: primer solution A and hardener II (catalytic reaction). The polymerization reaction occurs in the absence of air in a Teflon plate stationed between 0°C and -5°C for 24 h. Resin glue TECNHOVIT 3040 is used on the HISTOBLOCH Kulzer (Fig. 2 a-b).
CUT: semi-thin sections 2-3 mm with microtome.
STAIN: sections are stained with hematoxylin and eosin stain (HE) and by others histochemical techniques such as Periodic acid-Schiff's stain (PAS), Gomori, May-Grunwald-Giemsa, Unna-Pappenheim; it is also possible to use immunohistochemical techniques [10].
Dissection and histological observation permitted each embryo to be assigned to a specific Carnegie stage (Figs. 3-6).



a)



b)
Figure 2. a: Resin glue TECNHOVIT 3040; b: HISTOBLOCH Kulzer

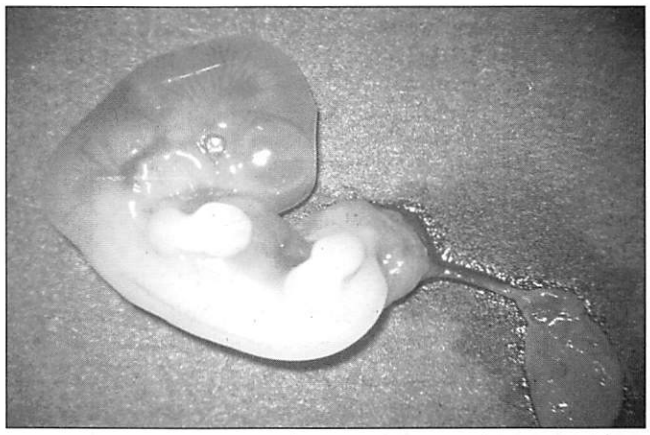


Figure 3. Embryo at stage XVII of the Carnegie classification

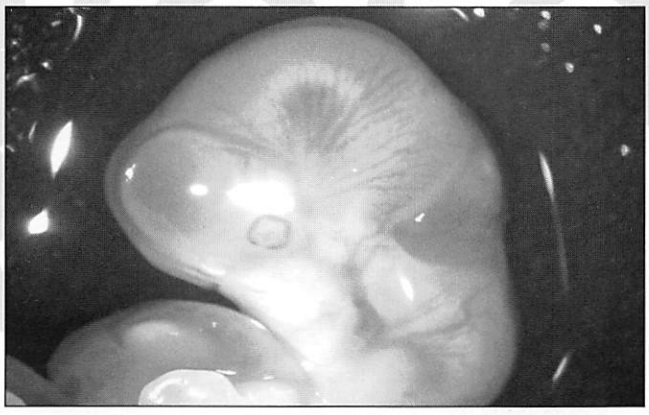


Figure 4. The Carnegie stage XVII. Pigmented lens placode

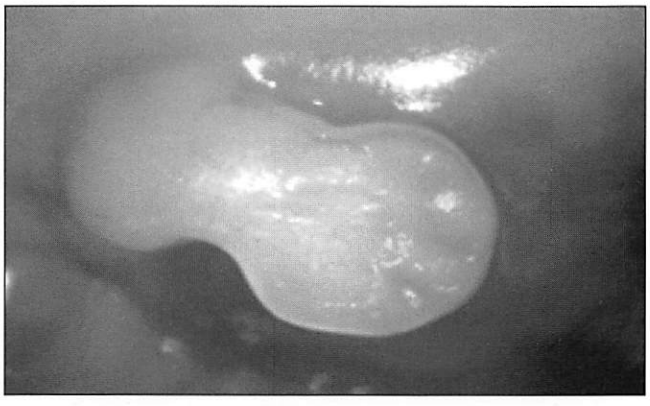


Figure 5. The Carnegie stage XVII. Formation of the interdigital zones

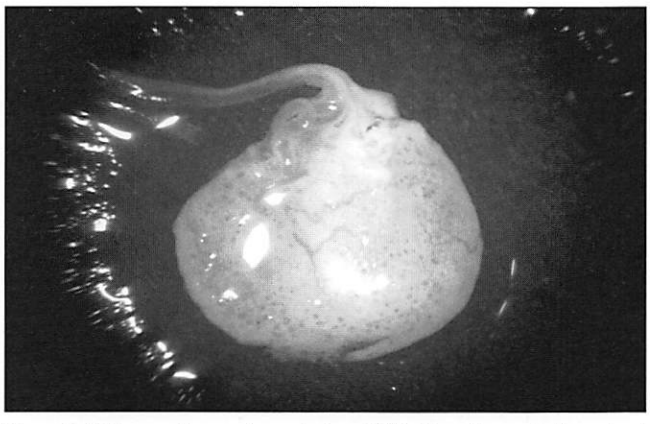


Figure 6. Yolk sac of an embryo at stage XVII. The microvesicular aspect and the vessels

Results

From 1994 to 2010 we examined 256 cases of first trimester spontaneous abortion that presented a gestational sac (Tab. 1).
The use of stereomicroscope dissection and the accurate histological studies using resin embedding techniques allowed us to distinguish the 256 cases into three subgroups: 114 gestational sacs with embryo, 38 gestational sacs with autolytic embryo or only the embryo appendages, 104 blighted ova (Fig. 7).

YEAR	EMBRYO	NO EMBRYO	AUTOLYTIC EMBRYO OR APPENDAGES	TOTAL
1994	7	18	1	26
1995	19	17	2	38
1996	9	5	4	18
1997	6	4	2	12
1998	5	4	2	11
1999	3	3	6	12
2000	5	2	1	8
2001	3	0	6	9
2002	10	3	0	13
2003	5	2	1	8
2004	5	5	4	14
2005	4	1	1	6
2006	7	11	1	19
2007	10	16	1	27
2008	3	5	3	11
2009	9	7	1	17
2010	4	1	2	7
TOTAL	114	104	38	256

Table 1.Our series since 1994

Gestational age at the time of pregnancy loss ranged from 6 to 13 weeks (mean gestational age: 10 weeks). Gestational age in women who underwent assisted reproduction ranged from 7 to 13 weeks (mean gestational age: 9 weeks) and there were no significant differences between the two groups.
The dating of growth arrest is extremely important. In the majority of cases the age of arrest is different from the age of the expulsion of the ovular chamber. This difference is important to identify the autolytic processes undergone by the embryo, that can be misleading in the identification of gross morphological abnormalities [11] (Fig. 8).
In our study we underline the importance of resin embedding for the precise application of the Carnegie classification leading to better diagnostic accuracy [12].

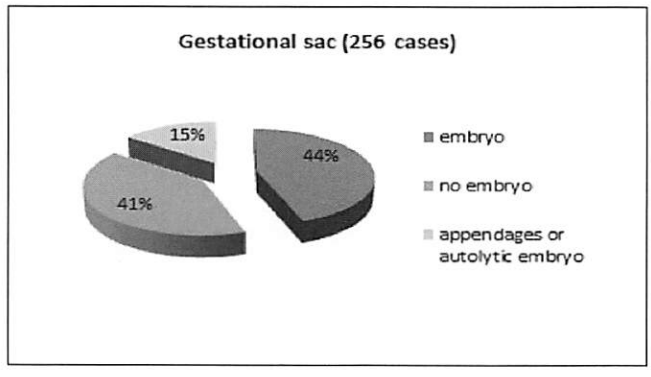


Figure 7. Reduction in the number of diagnoses of blighted ova and increasing the diagnosis of autolytic embryo or appendages

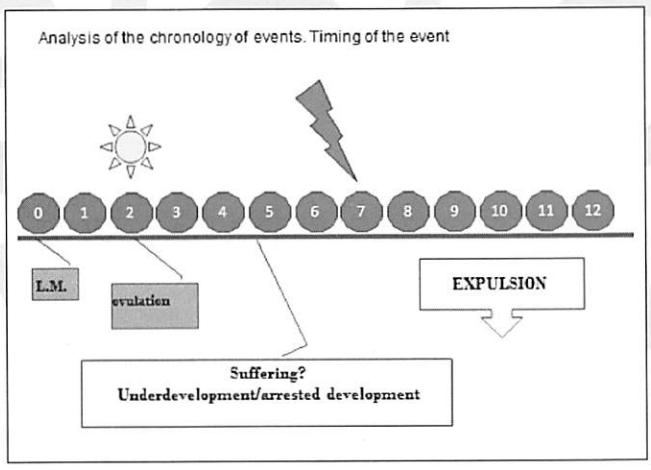


Figure 8. Chronology and timing of events.

In particular we were able to be more precise regarding the date of developmental arrest [13]. A diagnostic algorithm, constructed on the basis of our casistic composed of 114 embryos, distinguishes between the different moments in pregnancy loss: initial phase of embryonic suffering, actual moment of death, retention and expulsion. The distinction between these moments is the basis of our diagnostic approach (Fig. 9).

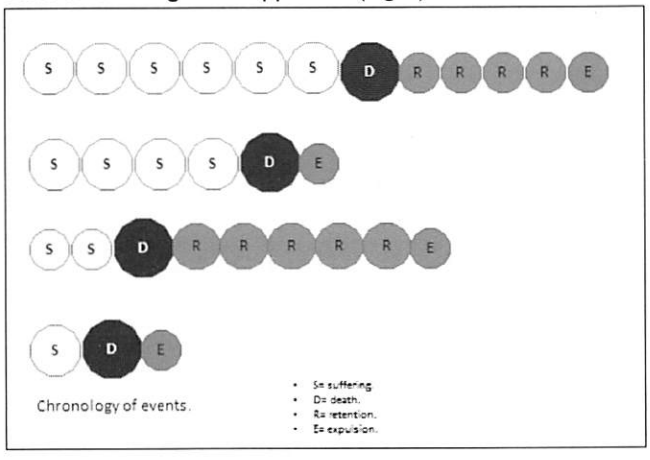


Figure 9. Diagnostic algorithm of different moments of pregnancy loss.

Discussion

In our experience thorough investigation using the aforementioned techniques permitted a significant reduction in the number of diagnoses of blighted ova, while increasing the number of diagnoses of autolytic embryo or embryo represented by only appendages [14].
The possibility of examining semi-thin sections in acrylic resin permits the study, in well-preserved embryos, of the expression of growth factor receptors as well as the migration of germ, hematopoietic and neural crest cells [15]. It also permits a better classification in case of partial autolysis with the definition of the stage of embryonic development according to the Carnegie classification [16]. This combined with an anatomical-clinical correlation and dialogue with patients and their families is helpful in identifying the causes that led to pregnancy loss. From the histological point of view we can detect abnormalities that may pave the way for investigations into the causes of pregnancy loss [17]. The determination of a detailed chronology of events, as well as the extent and severity of lesions, can help to define the role (if causal or casual) of possible external toxic agents.

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