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THE EXPLORATION OF THE HLA SYSTEM IN THE COUPLE INFERTILITY

***Soraya TEBIBEL[1]., Chahinez MECHATI[1]., Saber MESSAOUDI[1]., Fayçal NEDJAR[2].**

[1]Department of Animal Biology, University of Constantine 1, Algeria

[2]Laboratory HLA, C. H. U Ben Badis, University of Constantine 3, Algeria

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***Correspondence for Author:**

Dr. Soraya TEBIBEL,
Department of Animal Biology, University of Constantine 1, Algeria.
mounizidoune@hotmail.fr
sourayatebibel@yahoo.fr

ABSTRACT

To assess the impact of the HLA system on the couple infertility, a study is realized on 48 couples having presented or not spontaneous repeated abortions. The CROSS-MATCH and the HLA testing (class I and II) are carried out. The result is 9 CROSS-positive MATCH which 7 are accompanied with premature embryonic losses, which can mean that the woman develops antibodies anti paternal HLA. The development of these antibodies is not the only cause of immunological rejection, because in our analyzed population even if the CROSS-MATCH is negative there is always an abortion. In addition, the HLA testing of class II and I informs us about the presence of a HLA homology between parents; in this case, the mother does not consider her embryo (allogeneic) as foreign body. If the spontaneous miscarriages can be explained by classic causes (uterine anomalies, chromosomal and genetic abnormalities, the infectious causes, the hereditary or acquired thrombophilis, the immunological anomalies, the male causes and the environmental factors), some idiopathic and recurrent miscarriages are up to this day without adapted treatment.

Key words: pregnancy, HLA system, infertility, miscarriages, and spontaneous abortions to repetition.

1 INTRODUCTION

Because of its complexity and of the difficulty warning it, diagnosing and treating it, the infertility is a global problem of public health.

Infertility is defined by World Health Organization (WHO) as the inability of a couple to procreate or to carry a pregnancy to term at the end of two years of regular, unprotected sexual intercourse [1]. The repeated abortions occurring outside of the gynecological or hormonal causes can be observed among woman's having never had pregnancy conduct beyond three months or even in women who have already had a normal pregnancy. Many factors intervene probably to explain the immunological tolerance existing during the pregnancy, which makes that there is no rejection of the fetus. Among the factors that have been identified during *in vitro* of studies and in animals: the anatomical position of the fetus, the absence of expression at the level of the trophoblast of molecules of the system of histocompatibility HLA class I and II, the presence of blocking antibodies, a modification of the immune response, the production at the level of the fetus and the placenta of hormones and immunosuppressive substances [2].

Immunology of pregnancy involves many mechanisms to explain the paradox of the maintenance of the fetus within the maternal organism. The pregnant woman is not immunodepressed but it seems completely necessary that she recognizes the fetus as foreigner to develop the elements which contribute to protect him from mechanisms of rejection. This immunodeviation of woman towards active tolerance for her fetus is the result of the intervention of a cascade of non-specific or specific factors of paternal antigens of the fetus whose action is locally predominant in the peri-placental region. In certain circumstances, an anomaly or a deficiency of this harmonious balance of the organism can be responsible for a real abortive disease with an immunological origin.

2 MATERIAL AND METHOD

Our study (retrospective and prospective) is performed at the laboratory HLA -C. H. U-Constantine, on 48 couples to witch is realized:

- The lymphocytotoxicity.
- The CROSS- MATCH.
- The lymphocyte reaction mixed.

The data collection, established by a questionnaire, allowed us to record all the necessary information to our investigation from the couple itself. It includes:

The age of the woman; the age of the man; number of wedding years; number of children; number of abortions and the consanguinity.

2.1 The CROSS-MATCH

The cross-match allows the fast detection of preexisting, circulating cytotoxic antibodies, by a technique of lymphocytotoxicity complement dependent (reference technique). It is a test of compatibility between the woman serum and the cells of the spouse; it is obtained as follows:

-Isolation of mononuclear cells from the total blood of the man by Ficoll ;

-Deposit of mononuclear cells in plates of oiled TERASAKI;

-Injection of maternal serum;

-And then the classic lymphocytotoxicity (adding the supplement and of the vital dye) and even the reading will be the same principle (cell lysis).

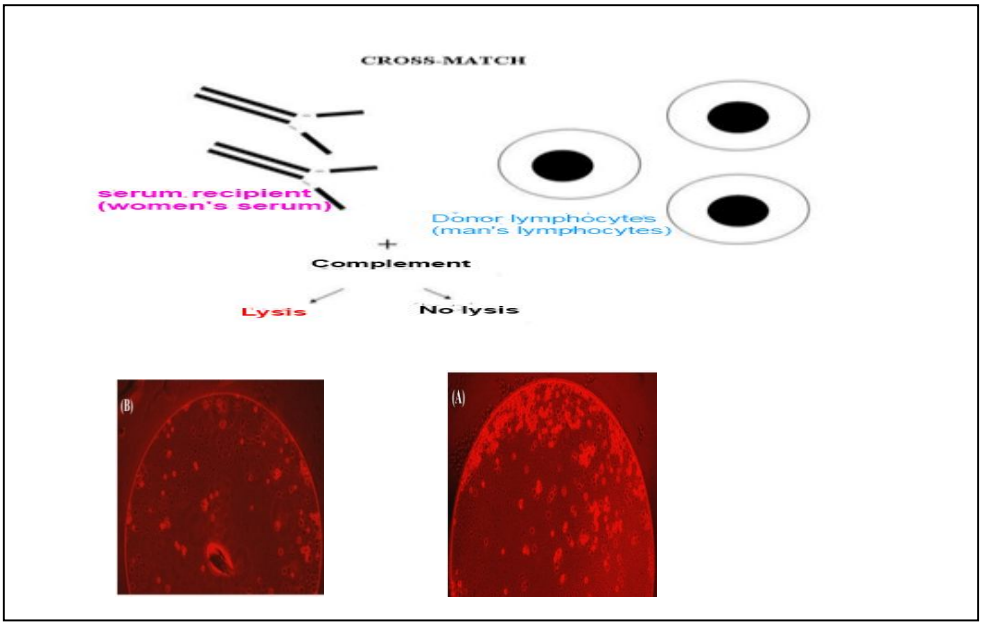

Fig.1: Principle of the CROSS-MATCH and sight under microscope.

2.2 The lymphocytotoxicity

Almost all laboratories adopt this technique, developed and introduced in 1964 by TERASAKI. It studies the anti-HLA antibodies and application in the in vitro transplant (CROSS-MATCH).

The principle of this technique is that; in the presence of the supplement, HLA cytotoxic antibodies can lead to the lysis of the lymphocytes bearing the corresponding HLA antigens, requiring:

- Antigens: lymphocytes suspension ;
- Antibodies: immune- anti-HLA serum;
- The Complement: serum of the rabbit.

The result is either negative when lymphocytes are alive, or positive when lymphocytes are lysed.

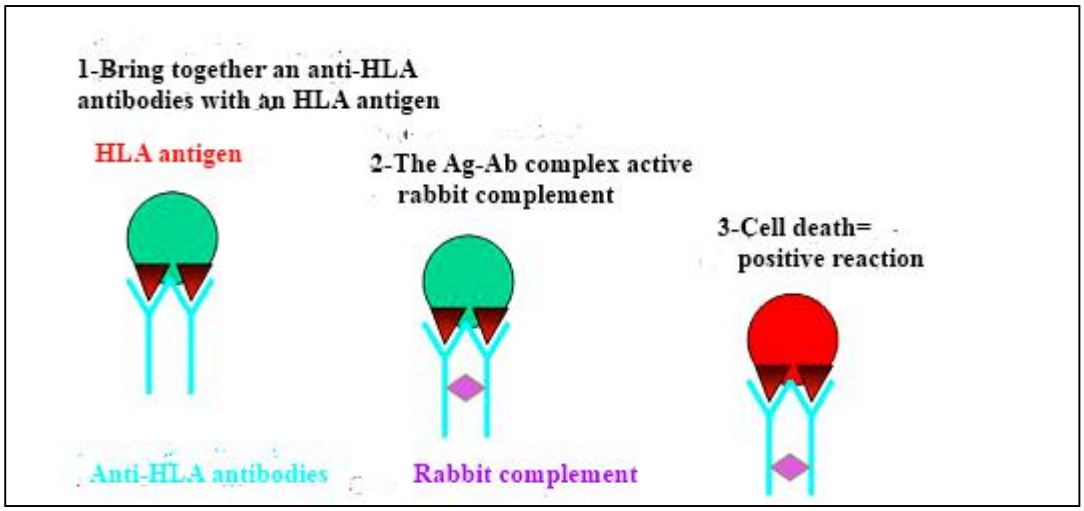

Fig.2: Principle of a positive reaction in lymphocytotoxicite.

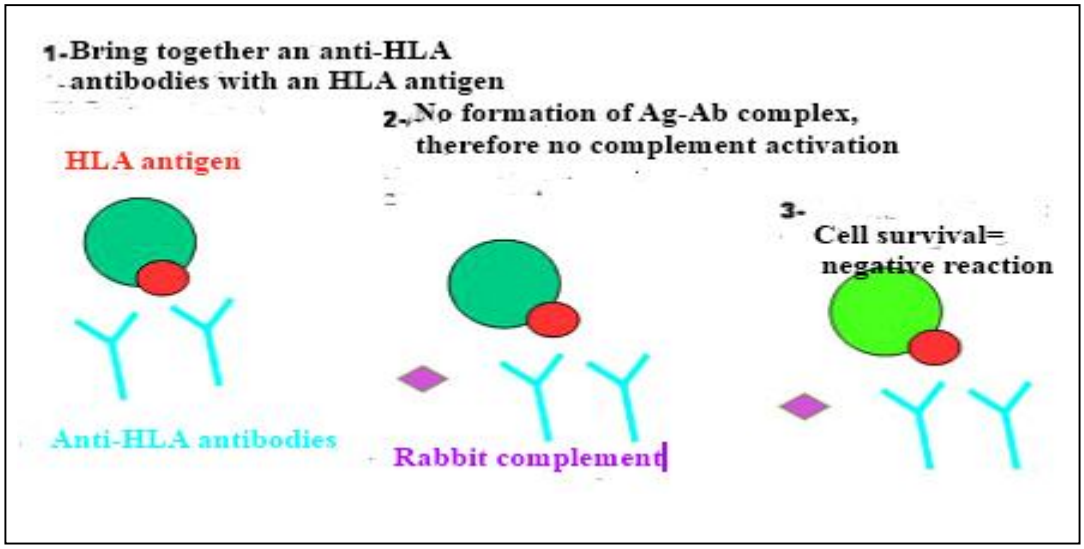

Fig.3: Principle of a negative reaction in lymphocytotoxicity.

2.3 Typing HLA (class I and II) by the technique of separation by FluoroBeads

The FluoroBeads B are immunomagnetic with a diameter less than1 µm, monoclonal antibodies anti-CD19 coupled with their surface are specific lymphocytes B.

The FluoroBeads T carry the antibodies anti-CD2 on their specific surface area of T lymphocytes.

From a sample of blood, the FluoroBeads provide a quick method of isolation of the complex ball-lymphocyte T or B using a magnetic separator.

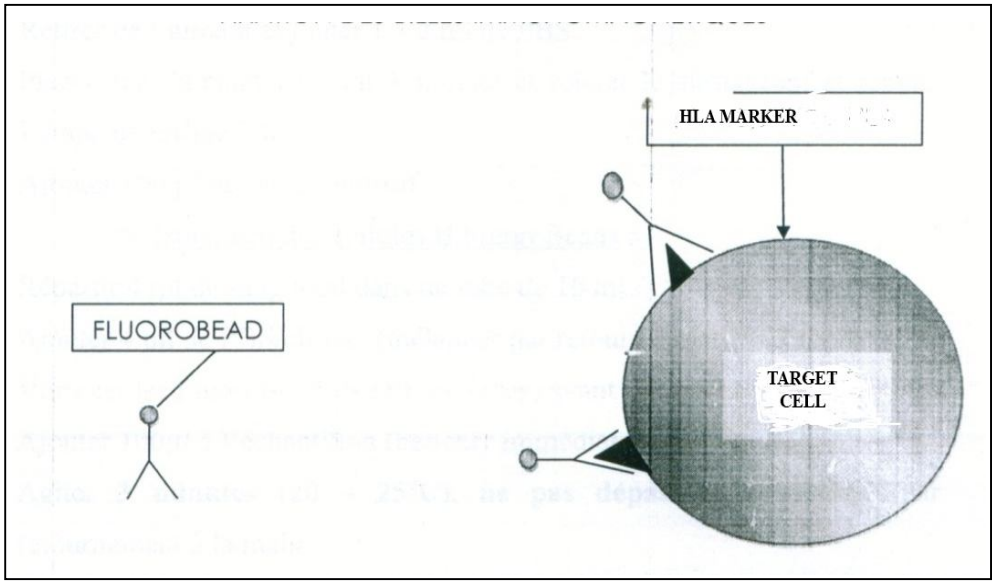

Fig.4: binding of immunomagnetic balls.

3 RESULTS

3.1 Old of the women

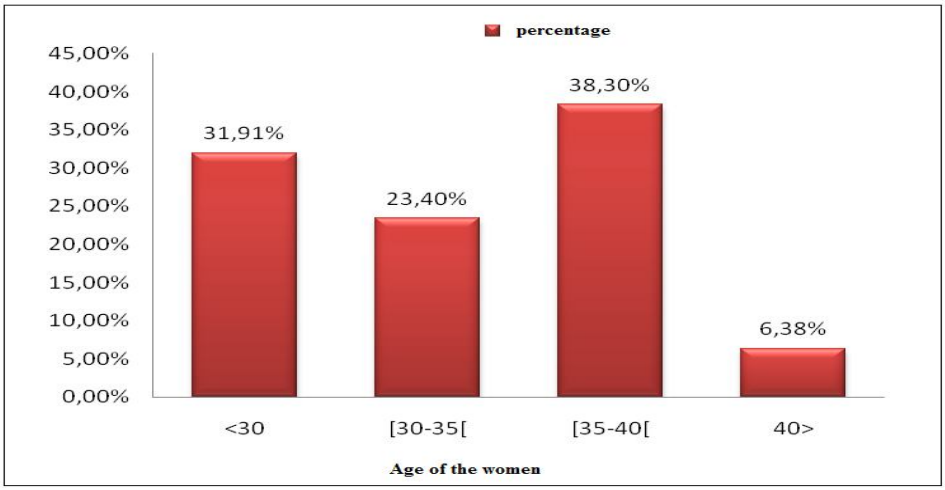

Fig. 5: Histogram representing the percentage of women having a problem of abortion according to the age bracket.

This histogram indicates the patients having had between 2 and 5 of the unexplained abortions; the distribution of the age groups of amplitude 5, shows the minimum age is 30 years and higher than 40 years for the maximum age. Our results reflect that the slices ranging between 35 and 40 years (with an average of 32.41) aer the women who worry much more theirs infertility.

3.2 Old of the mby

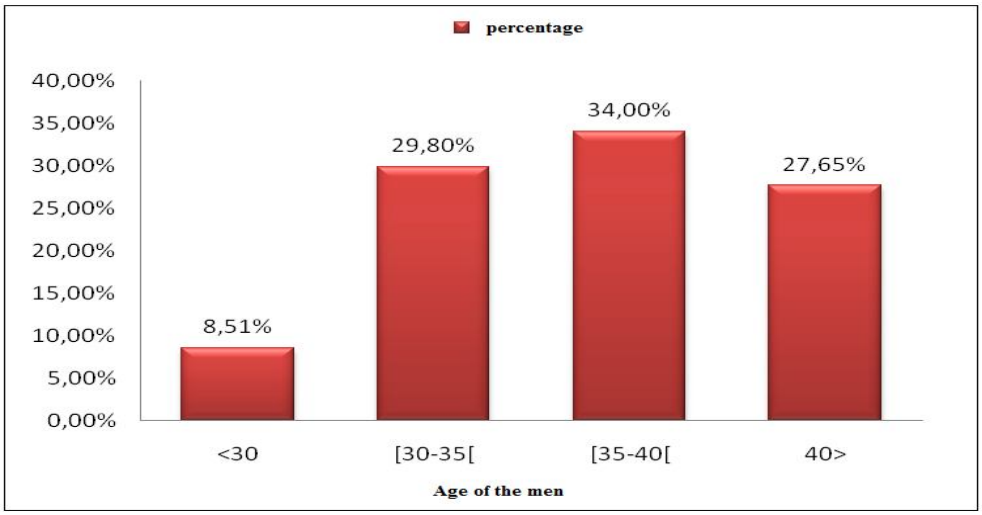

Fig.6: Histogram of the percentage of men consulting for a problem of fertility according to the age bracket.

The spouse is forgotten a little, that is an error since the male fertility is more and more often put in question.

Figure 6, distribution of the age brackets of the men of amplitude 5, indicates to us that the age groups ranging between 35 and 40 years (with an average of 34) are those, which consult for a problem of design.

This prevented us not from: that infertility affects the two sexes in the same way

3.3 Type of abortions

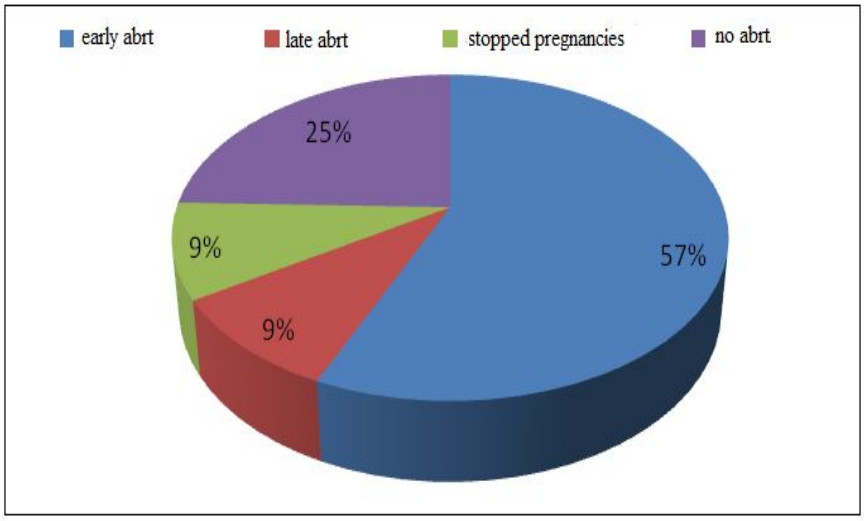

Fig.7: Sector of distribution of the percentage of tea abortions according to the type.

The data collection was made following questionnaires. Yew we analyze tea got results, it arises that on 48 women, 25% did not make abortions, 9% had stopped pregnancies, 9% of late abortions and 57% of early abortions (Fig.7).

The CROSS-MATCH carried out on the 48 couples, indicates 9 patients to us present a positive CROSS-MATCH and 38 reveal negativity.
On the 9 positive CROSS-MATCHES, 7 patients belong to the 57% i.e., the section representing of the early abortions iei.

Tea 2 negative CROSS-MATCHES are located:
- One in the portion of the late abortions.
- One in the portion of the stopped pregnancies.

3.4 Relationship between the number of abortion and the CROSS-MATCH

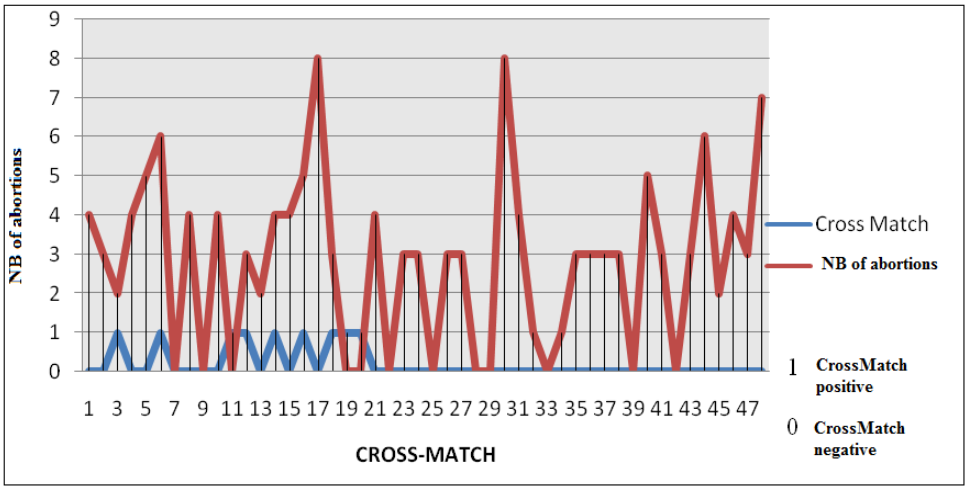

Fig.8: Correlation enters the number of abortions and the results of the CROSS-MATCH.

The examination of the individual curves enabled us to establish the relationship between the number of abortion and the result of the CROSS-MATCH (Fig.8).

It is the whole of the patients (48 couples), one notes 9 positive CROSS-MATCH what means that thesis 9 mothers development then came the meeting places visually analyzes a tissue anti HLA.

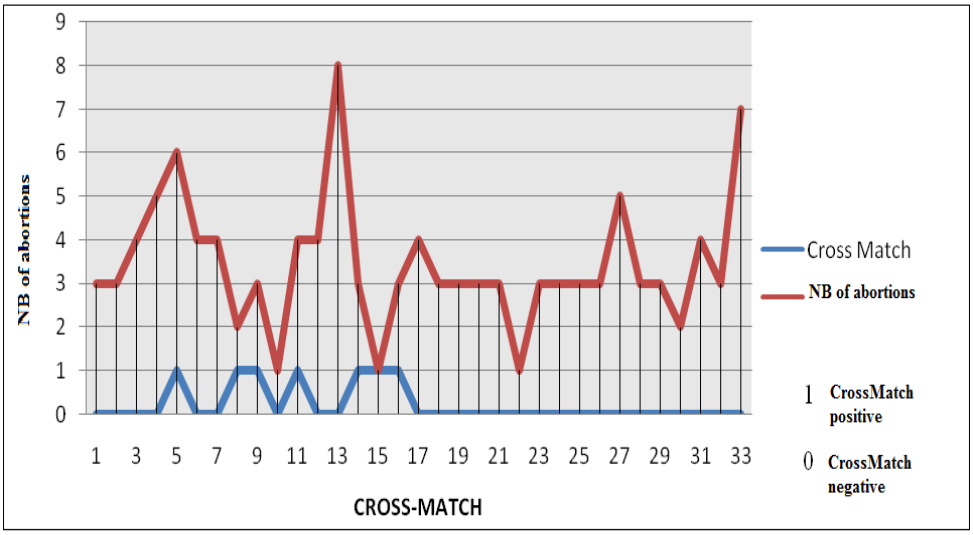

Fig.9: Correlation enters the number of early abortions and the results of the CROSS-MATCH.

Figure 9, indicates 33 women to us had unexplained early abortions with repetition, whose 7 CROSS-MATCH are positive (22 %) and 26 negative CROSS-MATCH (78 %).

In addition, in the tables of the data there are 10 consanguineous couples and present a negative CROSS-MATCH, therefore consanguinity does not influence the abortions in our sampling.

3.5 HLA testing of class I

In our sampling, 8 couples have been taken into account for the exploitation of the strong typing HLA class I of the woman and that of the man, the results are represented in the following table:

Table 1: Comparison of typing HLA class I of the mother and the father.

N° the couple	HLA testing of Class I of la♀	HLA testing of Class I of the ♂	Number of abortions
Couple 2	A1-A- / B7-B17	A10 (26) -A33 / B18-B22 (55)	3
Couple 3	A10-A- / B8-B18	A2-A- / B5 (51) -B12 (44)	2
Couple 4	A2-A3 / B14-B-	A1-A2 / B8-B14	3
Couple 5	A9-A- / B21-B-	A9 (23) -A- / B17-B21	5
Couple 8	A2-A- / B12(44.45) -B-	A11-A30 / B40-B70	4
Couple 10	A3-A- / B5 (51) -B18	A3-A- / B14-B40	4
Couple 12	A9 (24) -A- / B12(44.45) -B-	A30-A- / B18-B27 (73)	3
Couple 13	A1-A9 (23) / B5 (52) -B17	A9 (33) -A30 / B17-B-	2

The results show among the 8 couples studied, 4 couples (couples: 4, 5, 10 and 13) show a homology HLA (table.2), which represents 50% of couples taken into account.

Table 2: Couples presenting a homology HLA.

NO. The couple	Strong typing HLA class I of la♀	Strong typing HLA class I of the ♂	Number of abortions
Couple 4	**A2**-A3 / **B14**-B-	A1-**A2** / B8-**B14**	3
Couple 5	**A9**-A- / **B21**-B-	**A9**(23) -A- / B17-**B21**	5
Couple 10	**A3**-A- / B5 (51) -B18	**A3**-A- / B14-B40	4
Couple 13	A1-**A9**(23) / B5 (52)-**B17**	**A9**(33) -A30 / **B17**-B-	2

4 DISCUSSION

Miscarriages with repetition raise a problem of care because they remain unexplained in the majority of cases. The data obtained according to maternal and paternal age confirm on the whole that the assessment must be made when the woman is over 30 years and the man near 37years. The results of this study are in agreement with recent publications. When the biological age of the woman rises, there is a decrease in the quality of the uterine endometrium in which the embryo have to be implanted, it can also result from a lack of secretion of progesterone in luteal phase and the oocytes are more likely to present chromosomal abnormalities in their fertilized nucleus, what makes that there are more non-viable embryos.

It is also necessary to make sensitive the couples on the impact, now recognized, of the age of the man on the chances of conception. Thus, if 78 % of men conceive within 6 months when they are under 25 years of age, they are no more than 58.4 % to makes it beyond 35 years.

Recent works note that after 40 years, a decrease of the chances of pregnancy after intra-uterine insemination and an increase of miscarriages linked to the male age, as well as an increase of malformation syndromes and the different trisomies. The work presented at the European *Society of Human Reproduction and Embryology* in 2008, concerning more than 21239 cycles of intra-uterine insemination (with semen of the spouse), confirmes the effects of the age of both partners on the outcome of the insemination [3]. Our investigation took into account only patients, having had abortions with repetition of immunological presumed origin, from which the morphological and functional complete assessment of the couple has

beforehand eliminated any other possible cause of recurrent miscarriage. The request for the research of HLA immunization in pregnant women in a couple is proposed after three to four spontaneous abortions with repetition. The obtained data, on 48 achieved CROSS-MATCH, to couples confirm 9 positive. We can suggest that the blocking antibodies lead to the tolerance allografts; this may be due to the presence of paternal anti-HLA antibodies leading the decrease of the number of NK and Th lymphocytes (Killers and Helpers) and the rise of the number of lymphocytes suppressors. Besides, we notice that the false premature miscarriages (57 %) occurring during the first 15 weeks are significantly more frequent than those, called late (9 %), the second quarter (after the 15th week).

Among the studied population, the premature miscarriages, the tests reveal 7 positive CROSS-MATCH among the 9, thus when the woman develops paternal antibodies anti-HLA the embryonic rejection is immediate. On our sample, we realized a HLA testing of class I among 8 couples. We notice a homology among 4 couples (representing 50 %) in 1 or 2 markers HLA and presenting 2 to 5 abortions. The possibility of a failure of the mechanisms of maternal recognition was mainly discussed from the observation usual absence of anti-HLA antibodies in women presenting repeated abortions. Numerous works report that the number of HLA antigens shared by both partners seems upper in cases of repeated miscarriages than in control series. It is generally accepted that a certain degree of incompatibility is necessary to the recognition of the concept by the mother and to the development of a protective immune reaction during the pregnancy. As regards the homology, analyzed by the technique of class II HLA testing, the couple N°4 was tested and shows a semi-identity whose markers are DR1 and DQ1.

Most of the authors report that any homology in the HLA system leads to an excessive activity of the NK lymphocytes with rejection of the pregnancy. The cells of the fetus arn't in a direct contact with the maternel immune system because of the presence of a particular tissue, the trophoblaste. Thus, they can be neither identified as the non-itself nor destroyed by the maternal immune system. On the other hand, the trophoblaste is in contact with the maternal immune system but the characteristics of its membrane markers allow him to escape from the immune systems. Indeed, the absence of HLA classical marker suggests that these cells will not be recognized as the non-self. As for the antigen HLA-G, it allows them to escape the NK killer cells.

The presence of T cytotoxic cells was noticed at the woman's suffering from repeated abortions. However, it would be necessary to know if the appearance of cytotoxic cells is the cause of the foetal infringement or simply its consequence, because of the emergence of an inflammatory process in connection with a embryonic aggression of other origin. According to the work of Christiansen OB (1999), 94 women who have had repeated abortions and followed up regularly, those having the HLA DR1 and/or DR3 have 63% of risk to make an abortion, versus 29% for those not having those alleles. Other publications indicate that the DR1, DR3 and DR4 HLA markers are frequent among Caucasian woman's who have had spontaneous repeated abortions.

No association with repeated miscarriages has been found with the genes of HLA class I, including HLA-C; it is the example found at the woman of the couple N°4 presenting the DR1marker which may predispose them to ASR. The mechanism by which the alleles of the HLA class II confers a susceptibility on abortions could be a predisposition to the hypersecretion of certain cytokines (TNFα) at the foeto-maternal interface l [4]. To our knowledge, no study in Algeria addressed the role of the HLA system on idiopathic and recurrent abortions. However, our results don't provide enough information about maternal-fetal tolerance mechanisms and request a confirmation by prospective studies and/or by patients' even more important group.

CONCLUSION

Infertility is defined by the absence of pregnancy after two years of regular, unprotected sexual intercourse. For the same couple, the causes can be unique or multiple, feminine, masculine or very often mixed. The crossing of genetically different individuals' is the nature rule, suggesting that the materno-fœtale histo-incompatibility offers an advantage regarding reproduction. The facts are based on the observation that the couples with repeated implantations failures **show more HLA homology between them than couples with normal fertility**. The first ones having more chances to conceive a fetus identical to his mother, who would be unable to produce maternal blocking antibodies, which would lead to a failure of pregnancy. The presence of maternal antibodies directed against HLA paternal (CROSS-MATCH) also leads to a failure of the progress of the embryonic development.

However, the subject remains widely open, but requires a more deepened clinical and immunological research. In this domain, an inappropriate emphasis is often given to unproved hypotheses and to clinical studies poorly developed.

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