A comparison in concentration of heat shock proteins (HSP) 70 and 90 on chorionic villi of human placenta in normal pregnancies and in missed miscarriages

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Summary

Purpose: To investigate the role of heat shock protein (HSP) on the chorionic villi of human placental cells and to compare the concentration of placental HSP70 & 90 in term deliveries and in missed miscarriages.

Materials and Methods: Fifty products of conception from women who experienced first trimester missed miscarriage and 50 placentas from women who gave birth at term were studied. An immunohistochemical investigation was carried out with which we marked the localization of heat shock proteins 70 and 90 on the syncytiotrophoblastic, cytotrophoblastic, stromal and blood vessel cells, using specific antibodies which can detect the presence of those proteins on light microscopy. We compared their expression with the normal placental tissue of term pregnancies and with material acquired from first trimester missed miscarriages.

An indirect immunoperoxidase method was applied using polyclonal antibodies against HSP70 and HSP90 on formalin-fixed paraffin-embedded tissues.

Results: Expression of HSP90B was increased in chorionic villi of first trimester missed miscarriages concerning syncytiotrophoblasts, cytotrophoblasts, vessel and stroma cells compared to full-term placentas. There was a statistically significant increase of HSP90A expression in chorionic villi of first trimester missed miscarriages, concerning only the cytotrophoblast cells, compared to full-term placentas.

Expression of HSP70 cognate protein was significantly increased in chorionic villi of first trimester missed miscarriages, concerning syncytiotrophoblastic cells only, compared to full-term placentas.

Finally, HSP70 inducible protein was significantly increased in chorionic villi of first trimester missed miscarriages concerning syncytiotrophoblasts, cytotrophoblasts, vessel and stroma cells compared to full-term placentas.

Conclusions: The results of the present study have sufficiently shown that there is an increase of HSP70 & 90 expression in chorionic villi of first trimester missed miscarriages compared to full-term placentas and this increase may have an important implication on the miscarriage process.

Key words: First trimester missed miscarriage; Term placenta; Chorionic villi; Syncytiotrophoblast; Cytotrophoblast; Vessel; Stroma; HSP90B; HSP90A; HSP70 cognate; HSP70 inducible.

Introduction

The heat shock protein (HSP) belongs to an ubiquitous family of proteins that were initially described following cellular hyperthermia [1, 2]. The phenomenon of increased synthesis of HSPs on subjection to stress (not only thermal shock) has been known for 30 years, but their role has became more clear during the last few years. The HSP have been characterized as molecular chaperons [3] for the following reasons:

During the vital processes of protein biogenesis such as protein synthesis and translocation of proteins into different intracellular compartments, it is necessary for the protein to exist temporarily in an unfolded or partially folded conformation. As a result polypeptide regions that were buried before, now become exposed and can potentially interact with other active polypeptide parts and finally they can cause protein aggregation with catastrophic results for the cell. HSPs protect these interactive surfaces by binding to them and facilitating the folding of unfolded proteins, and consequently they prevent fatal protein aggregation [4]. The responsible genes for the HSP production are extremely resistant under stress conditions [5, 6].

HSPs are divided in different groups according to their molecular weight in Kilo Daltons. Thus the most common groups are HSP60, HSP70 and HSP90. These groups are subdivided in smaller groups based on some molecular characteristics. In our paper we are focusing on HSP70 cognate, HSP70 inducible [7], HSP90A and HSP90B [8]. Recent studies have revealed that HSPs play an essential role not only under stress conditions, but more commonly, they contribute to the cell regular homeostasis, as a part of the cellular answer to exogenous factors including oxidants, injury, surgery, and toxins [9, 10].

In humans, HSPs have been found in different tissues that underwent stress, like thyroid cells in autoimmune thyroiditis 11 and in atheromatous plaque 12.

Furthermore, recent studies that examined the level of HSP in human placenta and umbilical cord vessels

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suggest a fundamental role for HSP in the feto-placental unit 13.

Previous immunohistochemical studies revealed constant expression throughout the third trimester of pregnancy which does not change following the onset of labour, regardless of gestational age or premature labour 14. Their precise role on the fetomaternal unit still remains obscure.

Our effort was, by immunohistochemistry, to evaluate the expression of HSP70 and HSP90 on chorionic villi in cases of first trimester missed miscarriage and on the placentas of term pregnancies and to compare their concentration in order to acquire some information about the role of HSP in those two different groups. Furthermore, it is interesting to compare HSP expression on the chorionic villi in a missed miscarriage and the chorionic villi of a fully developed placenta at term after its passage through the genital tract.

Materials and Methods

Material included samples of conception products from 50 women who had missed miscarriages during the first trimester and 50 placentas from women who gave birth after 36 weeks of gestation in the community hospital of Larissa and from Helena Venizelou Hospital, Greece. All women in the first group underwent an evacuation of retained products of conception (ERPC). Criteria for the diagnosis of missed miscarriage were according to Nielson *et al.* [15] – an intrauterine sac with an embryo > 10 mm and without foetal heart activity on transvaginal scan. Their ages ranged from 18 to 39 years (mean 26.42). It was the first missed miscarriage for all of them. They originated from Greece (96%) and Albania (4%). All these women gave verbal consent to participate in the study. The full-term placenta group included 50 women of Greek origin (100%). Their ages ranged from 18 to 42 years (mean 26.03), parity was 1.44 (mean), and gestational age was 38.13 weeks (mean). The method of delivery included 28 normal deliveries (ratio 56%), 11 instrumental deliveries (ratio 22%) and 11 caesarean sections (ratio 22%). The reasons for the caesarean sections were previous caesarean sections (ratio 10%), two breech presentations at term (ratio 4%), three emergency caesarean sections for suspected foetal distress (ratio 6%) and two for failure to progress (ratio 4%). Twenty-eight of the newborns were females (56% ratio) and 22 were males (44% ratio). Specimens from these groups were submitted to the following procedure: tissues were fixed in 10% buffered neutral formalin for 24 hours. After fixation, tissues were dehydrated serially in 50%, 80%, 90%, 100% alcohol solutions and then in xylene. After dehydration the tissues were embedded in paraffin. After embedding 4 μ thick sections were cut with a microtome and then the sections were deparaffinised passing through xyline and alcohol solutions and washed with distilled water. Then the endogenous peroxidase activity was neutralized by immersing sections in 3% solution of H202 for 30 minutes. After washing with distilled water, the sections were immersed in tris-buffered saline pH: 7.6. The sections were then incubated in 10% normal rabbit serum in tris for 30 minutes in order to inhibit non-specific binding of the antibodies to the tissues. Then sections from each block were incubated with primary goat polyclonal antibodies HSP70 (K-20: cat # sc-1060), HSP70 (K-19: cat # sc-1059), HSP90A: (N-17: cat # sc-1055), HSP90B: (cat # sc-1057) purchased from Santa Cruz Co., CA, in a 1:50 tris solution, for two hours. Afterward the sections were rinsed for 5-6 min in tris buffer. Sections were incubated with rabbit anti-goat peroxidase conjugated antibody for one hour. After washing 5-6 min sections were incubated with DAB plus H202 for five minutes, then rinsed with tris buffer and distilled water, counterstained with hematoxylin, dehydrated and covered with per mount. All women signed an informed consent form to participate in the study.

Results

The grade of staining was classified as:

(–) negative when no stained cells were present, (±) weakly positive when less than 25% of cells showed weak staining, (+) slightly positive when all the cells showed slight staining, (++) moderately positive when all the cells showed moderate staining, and (+++) intensely positive when all the cells showed intense staining.

The type of staining in the positive cases was mainly diffuse cytoplasmic stain and to a lesser degree diffuse nuclear stain.

In the positive cases for HSP70 cognate protein, the stain was characteristically present on the free surface of syncytiotrophoblastic cells.

Concentration of HSP90B in syncytiotrophoblastic cells of missed miscarriage samples was slightly positive (+) in four cases, weakly positive (±) in 33 patients and

Figure 1. — HSP90B staining of chorionic villi of missed miscarriage cases. Note intense cytoplasmic staining of cytotrophoblasts (long arrow), weakly positive staining of syncytiotrophoblasts (short arrow) and moderate staining of stroma cells (arrow head) (x 450).

Figure 2. — HSP90B staining of chorionic villi of full-term placenta shows moderate staining of cytotrophoblastic cells (long arrow) and negative staining of the remaining elements (x 450).

Figure 3. — HSP90A staining of chorionic villi of missed miscarriage cases. Consecutive sections of villi of Figure 1 show slightly positive staining of cytotrophoblastic cells and negative staining of syncytiotrophoblast and stroma cells (x 450).

Figure 4. — HSP90A staining of chorionic villi of full-term placenta. Note weak staining of cytotrophoblastic cells (long arrow) and negative staining of the remaining elements (x 450).

Figure 5. — HSP70K19 cognate staining of chorionic villi of missed miscarriage cases in a cross-section showing only intense surface staining of syncytiotrophoblastic cells. All the other elements are negative (x 450).

Figure 6. — HSP70K19 cognate staining of chorionic villi of full term placenta showing only slight surface staining of syncytiotrophoblastic cells and negative staining of the remaining elements (x 450).

Figure 7. — HSP70K20 inducible staining of chorionic villi of missed miscarriage cases showing intense staining of syncytiotrophoblastic and cytotrophoblastic cells and moderate staining of stroma cells (x 450).

Figure 8. — HSP70 K20 inducible staining of chorionic villi of full-term placenta showing weak staining of cytotrophoblastic cells and negative staining of the remaining elements (x 450).

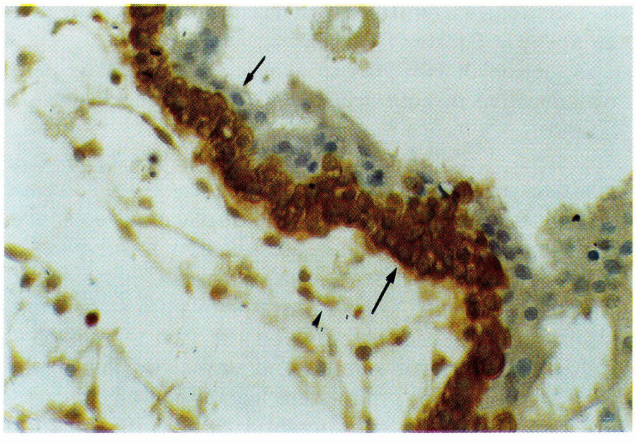

Fig. 1

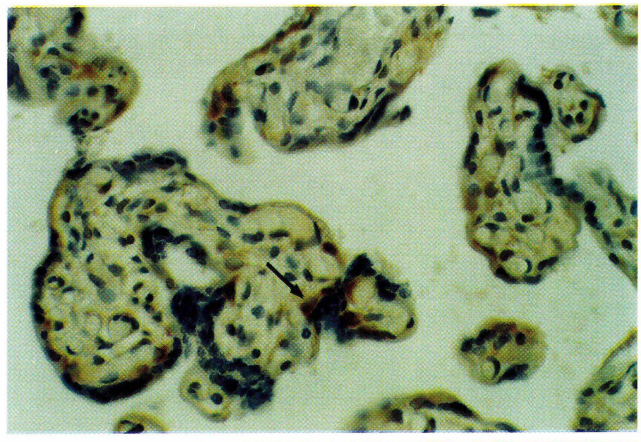

Fig. 2

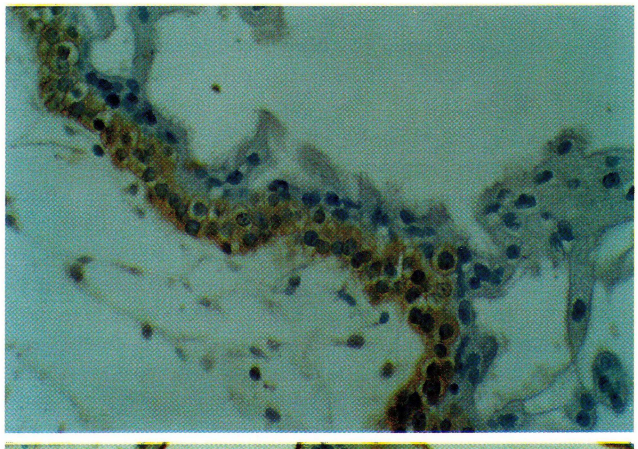

Fig. 3

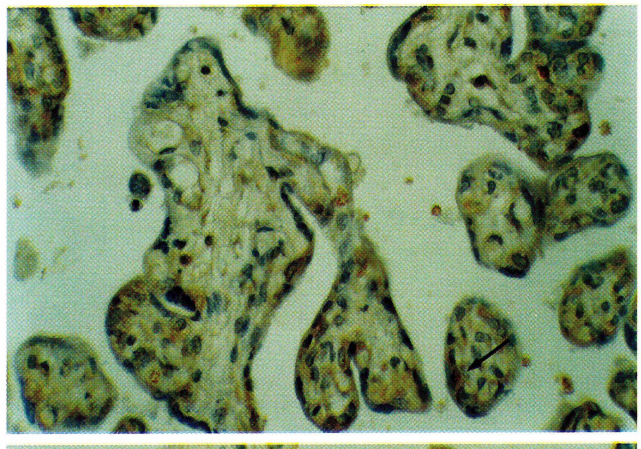

Fig. 4

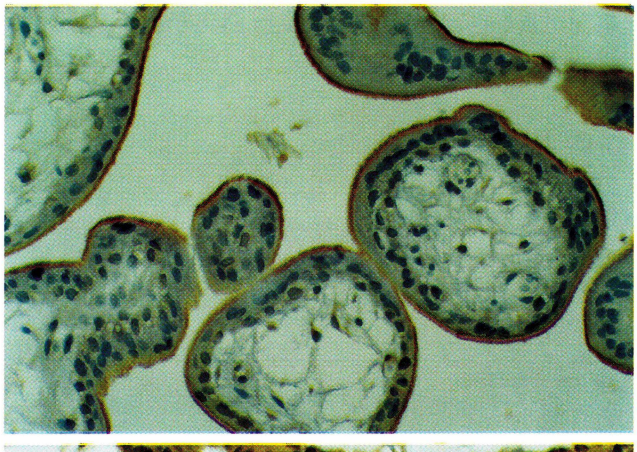

Fig. 5

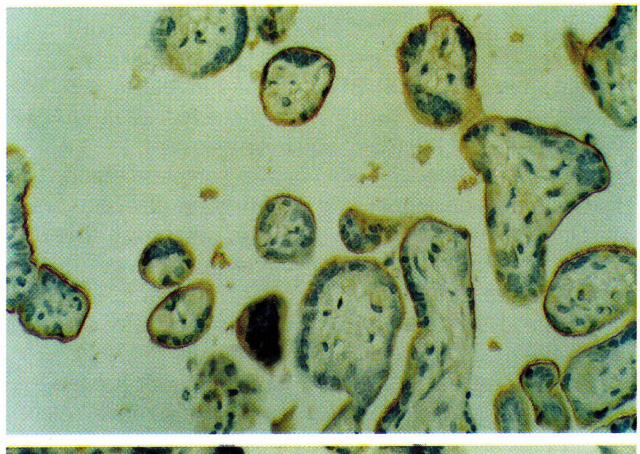

Fig. 6

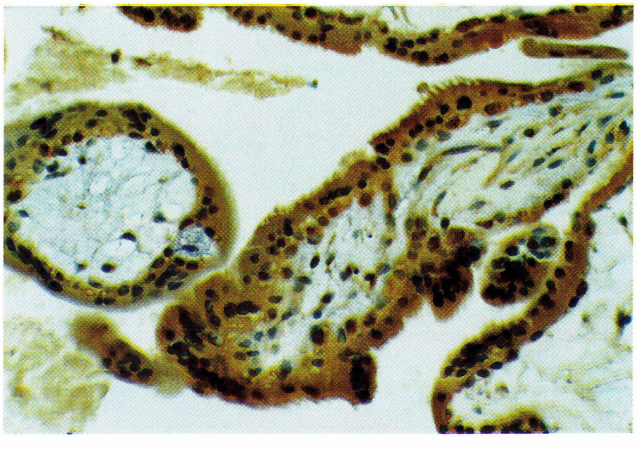

Fig. 7

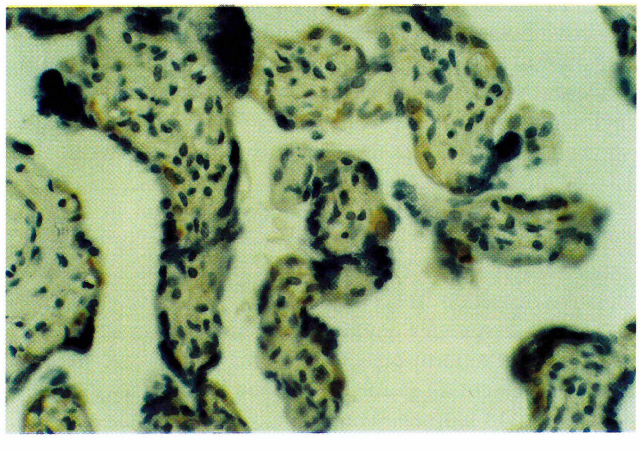

Fig. 8

Table 1.

HSP 90B	Missed Miscarriages	Term Placentas
Syncitiotrophoblasts		
+++	0	0
++	0	0
+	4	0
±	33	0
–	13	50
Cytotrophoblasts		
+++	14	0
++	27	6
+	9	14
±	0	26
–	0	4
Vessels		
+++	0	0
++	2	0
+	6	0
±	22	2
–	20	48
Stroma		
+++	0	0
++	2	0
+	6	0
±	17	2
–	25	48

negative (–) in 13 patients. On the other hand we could not detect any HSP90B concentration in syncytiotrophoblasts of term placental cells (50 cases). Using the Mantel-Haenszel test we found a statistically significant difference between the two groups ($p < 0.001$).

Concentration of HSP90B in cytotrophoblastic cells of missed miscarriage samples was detected as intensely positive (+++) in 14 cases, moderately positive (++) in 27 cases and slightly positive (+) in nine samples (Figure 1). HSP90B in cytotrophoblastic cells of term placentas was detected moderately positive (++) in six cases, slightly positive (+) in 14 samples, weakly positive (±) in 26 women and negative (–) in four women (Figure 2). Using the Mantel-Haenszel test we found a statistically significant difference between the two groups ($p < 0.001$).

Detection of HSP90B in missed miscarriage vessels gave us the following results: moderately positive detection (++) in two cases, slightly positive (+) detection in six cases, weakly positive detection (±) in 22 cases and negative (–) detection in 20 cases. Results for the term placental vessels were: weak detection (±) in two women and negative (–) in 48 women. The Mantel-Haenszel test showed a statistically significant difference between the two groups ($p < 0.001$).

Finally, we studied HSP90B concentration in stroma cells. In the missed miscarriage group the results were: moderately positive detection (++) in two cases, slightly positive detection in six cases, weakly positive detection (±) in 17 cases and negative detection (–) in 25 cases. Results for the term placental stroma cells were: weakly positive detection (±) in two women and negative detection (–) in 48 women. The Mantel-Haenszel test showed a statistically significant difference between the two groups ($p < 0.001$) (Table 1).

Detection of HSP90A in syncytiotrophoblastic cells was negative in both groups (50 cases in each group). HSP90A staining in cytotrophoblastic cells of missed miscarriages was slightly positive (+) in 11 cases, weakly positive (±) in 21 cases and negative (–) in 18 cases (Figure 3). HSP90 staining in term placental cells was slightly positive (+) in four cases, weakly positive (±) in eight cases and negative (–) in 38 cases (Figure 4) (50 cases). The Mantel-Haenszel test showed a statistically significant difference between the two groups ($p < 0.001$). Results for stroma and vessel tissues in the missed miscarriage group were negative in all 50 cases. On the other hand staining of HSP90A in placental tissues was weakly positive (±) in four women and negative (–) in 46 women for both stroma and vessel cells. Fisher's exact test showed no significant difference ($p < 0.059$) (Table 2).

Regarding the HSP70K19 cognate protein group, we had the following results: staining of syncytiotrophoblastic cells was intensely positive (+++) only on the surface in all 50 cases (Figure 5). In term placental villi syncytiotrophoblastic cell surface staining was slightly positive (+) in 15 cases, weakly positive (±) in 25 cases and negative (–) in ten cases (Figure 6). The Mantel-Haenszel test showed a statistically significant difference between the two groups ($p < 0.001$). Furthermore, we could not detect any HSP70K19 presence in cytotrophoblasts, stroma and vessel cells of both missed miscarriage and term-placenta groups (50 cases in each group) (Table 3).

Table 2.

HSP 90A	Missed Miscarriages	Term Placentas
Syncitiotrophoblasts		
+++	0	0
++	0	0
+	0	0
±	0	0
–	50	50
Cytotrophoblasts		
+++	0	0
++	0	0
+	11	4
±	21	8
–	18	38
Vessels		
+++	0	0
++	0	0
+	0	0
±	4	0
–	46	50
Stroma		
+++	0	0
++	0	0
+	0	0
±	4	0
–	46	50

Staining of HSP70K20 inducible protein revealed: expression of HSP70K20 in syncytiotrophoblastic cells of missed miscarriage samples was detected intensely positive (+++) in eight cases, moderately positive (++) in six cases, slightly positive (+) in nine samples, weakly positive (±) in 13 cases and negative (–) in four cases (Figure 6). In term placental villus syncitiotrophblastic cell staining was slightly positive (+) in four cases, weakly positive (±) in 36 cases and negative (–) in ten cases. Using the Mantel-Haenszel test found a statistically significant difference between the two groups ($p < 0.001$).

Expression of HSP70K20 in cytotrophoblastic cells of missed miscarriage samples was detected as intensely positive (+++) in 14 cases, moderately positive (++) in 25 cases, slightly positive (+) in nine samples, and weakly positive (±) in two cases (Figure 7). In term placental cells HSP70 expression in cytotrophoblastic cells was slightly positive (+) in four cases, weakly positive (±) in 37 cases and negative (–) in nine cases (Figure 8). Using the Mantel-Haenszel test a statistically significant difference was found between the two groups ($p < 0.001$).

HSP70K20 concentration in missed miscarriage vessel cells was: intensely positive (+++) in 12 cases, moderately positive detection (++) in seven cases, slightly positive (+) in 11 cases, weakly positive (±) in 14 cases and negative (–) in six cases. Results for the term placental vessel cells were slightly positive (+) in two women, weakly positive (±) in 24 women and negative (-) in 24 women. The Mantel-Haenszel test showed a statistically significant difference between the two groups ($p < 0.001$).

The results for the HSP70K20 group for its expression in missed miscarriage stroma cells was intensely positive (+++) in 18 cases, moderately positive (++) in two cases, slightly positive (+) in ten cases, weakly positive (±) in 14 cases and negative (–) in six cases. Results for the term placental stroma cells were weakly positive (±) in 24 cases and negative (–) in 26 cases. The Mantel-Haenszel test showed a statistically significant difference between the two groups ($p < 0.001$) (Table 4).

Table 3.

HSP 70 K19	Missed Miscarriages	Term Placentas
Syncitiotrophoblasts		
+++	50	0
++	0	0
+	0	15
±	0	25
–	0	10
Cytotrophoblasts		
+++	0	0
++	0	0
+	0	0
±	0	0
–	50	50
Vessels		
+++	0	0
++	0	0
+	0	0
±	0	0
–	50	50
Stroma		
+++	0	0
++	0	0
+	0	0
±	0	0
–	50	50

Table 4.

HSP 70 K209	Missed Miscarriages	Term Placentas
Syncitiotrophoblasts		
+++	8	0
++	6	0
+	9	4
±	13	36
–	4	10
Cytotrophoblasts		
+++	14	0
++	25	0
+	9	4
±	2	37
–	0	9
Vessels		
+++	12	0
++	7	0
+	11	2
±	14	24
–	6	24
Stroma		
+++	18	0
++	2	0
+	10	0
±	14	24
–	6	26

Discussion

HSP expression is a unique cell response to stress conditions [10]. Missed miscarriage is a situation of acute stress for the pregnancy products and childbirth is a stressful condition for the fetoplacental unit. Our effort was to investigate the role of HSP in these acute stress conditions and compare the expression. The results of the present study clearly demonstrate that there are qualitative and quantitative differences in the expression of HSP70 and HSP90 in the chorionic villi of missed miscarriages and full-term births.

Concerning the qualitative differences it is worthwhile to note that the expression of HSP70 cognate was present on the free surface of the syncytiotrophoblast and this may suggest an important role for this protein in to the transportation of vital substances between maternal blood and the fetoplacental unit. Concerning the quantitative differences it was shown from the above results that HSP90A, HSP90B, HSP70 inducible and cognate type, were strongly expressed in the missed miscarriage group in comparison with the term placenta group. With the exception of HSP90A expression in syncytiotrophoblasts, vessels and stroma cells which showed no significant dif-

ferences between the two groups, all the other HSP families that were studied showed that they have a much more intense expression in the cells of missed miscarriage cases. Our study is one of the few studies demonstrating HSP expression in human chorionic villus cells. One might expect HSP expression to be much higher in term placenta cells because of the magnitude of stress which is much higher in normal labour, but our study showed the opposite. Due to these significant differences in HSP expression, it could be suggested that HSP90A, HSP90B and HSP70 play a significant role in the miscarriage process by interfering with placenta function. Despite our efforts it is still the beginning of such an assumption and there are many other aspects that have to be explored, such as the presence of a fetal anomaly (chromosomal or somatic), the degree of the stress and the correlation to HSP expression. We hope that in the future further investigations will shed more light on the role of HSP expression in the process of miscarriage and full-term pregnancy.

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