DOWNLOAD PDF HLA-G EXPRESSION DURING HUMAN PREIMPLANTATION EMBRYONIC DEVELOPMENT

Chapter 1 : Down-regulation of HLA-G Attenuates Cleavage Rate in Human Triploid Embryos

HLA-G expression during preimplantation human embryo development. A Jurisicova, R F Casper, N J MacLusky, G B Mills, and C L Librach Department of Obstetrics and Gynecology, Toronto Hospital Research Institute, ON, Canada.

Advanced Search Abstract Improved culture conditions that support the development of human embryos to the blastocyst stage in vitro led to the prospect of blastocyst transfer to increase pregnancy rates. Thus, there is a need for characterization of possible biochemical markers able to predict the implantation potential of human blastocysts. No association could be found between the amount of transcript for these genes, total cell number or cell death rate. SP-1 protein concentrations were assessed in the culture medium of blastocysts using enzyme-linked immunosorbent assay. There was a significant positive correlation between SP-1 concentrations and blastocyst cell numbers. Moreover, synthetic oviductal medium enriched with potassium resulted in an SP-1 concentration twice as high as that observed using human tubal fluid medium. These data suggest that SP-1 may be used to select blastocysts with higher cell number, possibly resulting in higher pregnancy rates. New knowledge on metabolic requirements of the preimplantation embryo and improvement of culture media allowed extended in-vitro embryo culture up to the blastocyst stage. A series of preliminary trials of blastocyst transfer in selected groups of patients appeared to result in excellent pregnancy rates for reviews, see Bavister and Boatman, ; Desai, However, as with any change applied to a routine clinical setting, the transfer of embryos at this later developmental stage has several pitfalls and benefits that should be considered Gardner and Schoolcraft, ; Tsirigotis, This observation is further supported by observations of morphological Dokras et al. Furthermore, cellular abnormalities such as lack of inner cell mass ICM cells Winston et al. These results prompted us to analyse the quality of human blastocysts with respect to their transcriptional activity. Both of these markers are used for assessment of functionality as well as chromosomal normality of the developing placenta and embryo Bogart et al. Moreover, both these markers have been detected at variable levels in culture media of human embryos Dimitriadou et al. HLA-G is a non-classical class I antigen with low polymorphism, found on the surface of invasive cytotrophoblast cells that are in direct contact with maternal decidua Kovats et al. Patients who elected not to freeze their spare embryos for future transfers either by choice or because the embryos were not suitable for cryopreservation due to extensive fragmentation or multinucleation of blastomeres, were asked to donate these embryos for research. This project was approved by the human ethics committee of the Toronto Hospital and by the University of Toronto. Spare embryos of variable quality that appeared to arise from normally fertilized oocytes with two pronuclei 2PN , as well as abnormally fertilized 3PN or activated oocytes 1PN , were used in this study. Assessment of embryo quality and developmental stage were recorded daily until the embryos reached the expanded blastocyst stage. The assessment of cell death was based on DNA condensation and nuclear morphology Jurisicova et al. The cell death index CDI was calculated as the percentage of total cells which exhibited nuclear abnormalities. After 30 min incubation, the zona pellucida was removed and embryos were viewed briefly under fluorescence. The numbers of normal cells and cells with condensed DNA were recorded. Subsequently, these randomly chosen blastocysts were used for analysis of gene expression. All embryos analysed in this group were cultured in HTF medium. The amplified material was dot blotted and analysed by hybridization with radiolabelled cDNA probes, followed by quantification of signals on a phosphorimager. Results are presented in counts per minute c. Estimates of mRNA copy number were calculated as described Rambhatla et al. Excess sites were blocked with phosphate buffered saline PBS , 0. The effect of culture conditions, abnormal nuclear morphology and ploidy on total cell number and the cell death index were analysed by the Mannâ€”Whitney rank sum test and by Kruskalâ€—Wallis one-way analysis of variance on ranks respectively. Of these embryos, contained at least one multinucleated blastomere and 16 contained blastomeres with fractured nuclei. All the abnormally fertilized or activated oocytes were obtained by ICSI. One hundred and seventy of them appeared to have only a single pronucleus 1PN with two polar

bodies, while had three visible pronuclei 3PN. The type of culture medium used affected the rate of blastocyst formation of 2PN embryos Table I. A significantly higher proportion of normally fertilized embryos developed to blastocyst stage in KSOM medium 25 versus In contrast, no effect of medium on the rate of blastocyst formation was observed in 1PN 7. Interestingly, none of the embryos containing blastomeres with fractured nuclei developed beyond the cell stage. All blastocysts were subjected to analysis of cell number, but five were lost during this procedure. Occasionally necrotic cells 0. Some differences in cell number and cell death index were observed among blastocysts developed from normally 2PN and abnormally 1PN or 3PN fertilized embryos. Blastocysts that originated from activated oocytes 1PN appeared to have a higher incidence of cell death than normally fertilized embryos. However, due to the small sample size, these differences did not reach statistical significance see Table II. As expected, a great variability in expression of HLA-G was also observed. There was no relationship between transcript accumulation for either of these genes and cell number or CDI. Analysis of SP-1 concentrations in culture medium In order to investigate a possible association between cell number and cumulative secretion of embryonic markers such as SP-1, an ELISA assay was performed using an SP-1 specific antibody. Medium from 92 blastocysts was analysed, out of which 80 originated from normally fertilized oocytes and 12 from 3PN zygotes. Interestingly, in 14 blastocysts SP-1 was undetectable. Discussion The developmental potential of early cleavage stage human embryos obtained by IVF is variable as later reflected in the quality of obtained blastocysts. In order to increase the chance of a successful pregnancy, the transfer of embryos at the blastocyst stage coupled with selection of the most viable embryos is desirable. However, at the present time, our knowledge of suitable biochemical markers that could be used to predict viability of blastocysts is extremely limited. In-vitro development past the four-cell stage is believed to be one of the factors allowing selection of embryos that may reach the blastocyst stage. Even though this pool represents a group of developmentally compromised embryos, gene expression analysis of this subset can offer at least some information regarding mechanisms governing molecular events occurring during human preimplantation embryo development. The rates of blastocyst formation observed in this study are within the reported range of development for spare embryos. Cell numbers and CDI, recorded at day 6 and 7, were comparable with those previously reported Hardy et al. Interestingly, a positive effect of KSOM media on the frequency of blastulation in the group of normally fertilized embryos was observed. In addition, KSOM supplemented with non-essential amino acids stimulated the ability of embryos to secrete SP-1, since concentrations almost twice as high were recorded in this medium when compared to HTF. Thus, KSOM not only improves the developmental potential of human embryos, but its composition also appears to have a positive influence on the rate of metabolism. This observation is in agreement with that of a previous study Ho et al. Cell death appears to be an integral component of development of mammalian embryos at the blastocyst stage for review see Hardy, Results of several experiments published recently identified some triggers of apoptosis in mouse and rat blastocysts, such as hyperglycaemia Moley et al. Unfortunately, the molecular triggers of this process in the human remain unknown. In the current study, expression was detected as early as at the two-cell stage, which could be attributed to the more sensitive RT-PCR technique employed. Previously, it was proposed that the trophoectodermal TE lineage in humans is established by a single cell formed after the second cleavage division Edwards and Beard, Moreover, based on experiments performed with X inactivation in the mouse Sheardown et al. In a subset of cells in eight-cell embryos, Oct-4, a transcriptional repressor of placental markers Liu and Roberts, , appears not to localize to the nucleus and thus fails to repress the TE state reviewed in Edwards and Beard, Oct-4 transcript also could be detected in human oocytes and early cleavage-stage embryos Abdel-Rahman et al. Consistent with this hypothesis is our observation that HLA-G antigen could be found on the surface of all blastomeres of two- to eight-cell human embryos, but at the blastocyst stage only TE cells maintain this expression Jurisicova et al. Moreover, embryos maintained in favourable culture conditions, such as on co-culture with Vero cells, secrete higher concentrations of HCG Turner and Lenton, At the present time, it is not clear whether lack of transcription, translation or secretion is responsible for such variability. This could be explained either by the dilution by the

larger volume of media used to culture embryos 1 ml or insufficient sensitivity of the assay. This observation is consistent with our previous report Jurisicova et al. Originally, it was proposed that HLA-G may be a human homologue of the murine Ped gene that appears to influence the rate of mouse embryo cleavage. In a recent study Saith et al. In this study, a significant positive correlation was found between cell number and concentrations of SP This observation may have clinical relevance, since this molecule can be used as a marker for selecting blastocysts with higher cell numbers. However, the real predictive value of SP-1 with respect to developmental potential needs to be evaluated by transfer of such blastocysts and prospective analysis of pregnancy outcome. Developmental potential of human embryos cultured in human tubal fluid HTF or synthetic oviductal medium supplemented with potassium KSOM in vitro Medium.

Chapter 2 : HLA-G expression during preimplantation human embryo development. - CORE

Interestingly, expression of HLA-G mRNA was associated with an increased cleavage rate, as compared to embryos lacking HLA-G transcript. Thus, HLA-G could be a functional homologue of the mouse Qa-2 antigen, which has been implicated in differences in the rate of preimplantation embryo development.

Advanced Search Abstract Human leukocyte antigen HLA -G is a major histocompatibility gene expressed almost exclusively in extravillous trophoblasts at the fetalâ€"maternal interface. HLA-G may play a role in protecting the fetus from attack by the maternal natural killer cells. The extravillous trophoblasts invade the decidua and maternal spiral arteries. The factors which regulate the cell-specific expression of HLA-G are unknown. In this study we asked if HLA-G is expressed in extravillous trophoblasts that develop outside of their normal cellular environment, as in the case of ectopic pregnancies. Since all ectopic pregnancies implant in the absence of underlying decidua we also used a placenta accreta as an experimental control. In a case of placenta accreta which develops without decidua basalis and is therefore adherent to the underlying myometrium , HLA-G mRNA and protein were also expressed. These results suggest that HLA-G expression is induced in a cell autonomous manner rather than determined by appropriate environmental cues. In the placenta, throughout the three trimesters of pregnancy, HLA-G is found in the invasive extravillous trophoblast cell lineage Yelavarthi et al. Extravillous trophoblasts are epithelial cells derived from the trophoectoderm of the developing blastocyst. They form cell columns which anchor the placenta to the uterus and invade the decidua interstitial invasion and the maternal spiral arteries endovascular invasion. Expression of HLA-G is related to the degree of invasiveness of the trophoblasts, as was observed both in vivo and in vitro. In addition, when primary trophoblasts are isolated and cultured, HLA-G expression increases as the cells differentiate along the invasive pathway McMaster et al. When invasion is defective and shallow as in the toxaemia of pregnancy, pre-eclampsia, protein and mRNA expression of HLA-G is reduced both in vivo and in isolated trophoblasts in vitro Hara et al. These studies suggest a strong positive correlation between the invasive phenotype of trophoblasts and HLA-G expression. Furthermore, HLA-G expression is not found in the non-invasive syncytiotrophoblast of the chorionic villi McMaster et al. Although in direct contact with maternal blood and tissue, invasive trophoblasts are not rejected by the mother. Trophoblasts, of fetal origin, should theoretically be rejected by the mother as hemiallografts. But all trophoblasts, villous as well as extravillous, lack expression of the highly polymorphic HLA class I antigens A and B Redman, ; Bulmer and Johnson, ; reviewed in Christiansen, ; Fernadez et al. Lack of HLA expression by trophoblasts could explain why the maternal T cells do not recognize trophoblasts as foreign. However, NK natural killer cells are the major lymphocyte population at the fetalâ€"maternal interface. The invasive trophoblasts encounter the maternal NK cells when they invade the uterine tissue. Expression of HLA-G by invasive trophoblasts may afford protection from attack by the maternal NK cells in the uterus. The significance of these results with respect to the situation in the placenta is unknown. These studies lend support to the notion that HLA-G expression in invasive trophoblasts may protect these fetal cells from maternal immune surveillance. Attempts have been made to reveal elements in the promoter of HLA-G which govern its restricted expression pattern but no specific DNA sequences which confer the unique transcriptional regulation have yet been found Schmidt et al. However, the elements which govern the transcriptional regulation of classical major histocompatibility complex MHC class I promoters are well characterized Israel et al. Although specific factors which control the tight transcriptional regulation of HLA-G are not yet known, they may be induced by appropriate environmental cues which are unique to the maternalâ€"fetal interface. Alternatively, expression of HLA-G may be an intrinsic property of the state of differentiation of the extravillous trophoblast cell lineage. This fundamental question in developmental biology, of whether environment or cell autonomy induces gene expression, is rarely amenable to study in the human system. We were able to address this question for HLA-G by studying specimens from 13 ectopic pregnancies as well as in a placenta accreta used

as a control. Materials and methods Specimen collection A paraffin-embedded block from a placenta accreta was selected from the archives of the Pathology Department at Hadassah Hospital, Mount Scopus. A placenta accreta develops without decidua basalis and is therefore adherent to the underlying myometrium. Normal first trimester placentae were taken from elective pregnancy terminations all in accordance with the protocol of the Human Subjects Committee approved by our institution. Reports of cases of ectopic pregnancies were detected in a medical records search at Mount Scopus Hospital, covering the past 6 years. We selected ectopic pregnancies in which a heartbeat had been detected by ultrasonography to obtain samples of presumed healthy tissue for in-situ hybridization analysis which requires intact mRNA. To detect extravillous trophoblasts the cells which normally express HLA-G , we performed immunohistochemistry for cytokeratin on the paraffin-embedded sections. The slides were examined by a perinatal pathologist and those specimens with extravillous trophoblasts were chosen for in-situ analysis. To obtain further samples we expanded our search parameters and examined 40 ectopic specimens by cytokeratin immunohistochemistry and selected those with extravillous trophoblasts. In total we characterized through mRNA in-situ hybridization analysis 13 ectopic specimens for HLA-G expression, seven with heartbeats and six without. We added an antigen retrieval microwave protocol Copeman et al. No expression was detected in the syncytiotrophoblast nor in the villous cytotrophoblasts. Hybridization with an HLA-G sense riboprobe did not reveal hybridization above background levels, thus confirming our probe specificity Figure 2A and B. Trophoblasts in ectopic pregnancies Table I summarizes the clinical profile of the patient cohort. The ultrasound findings record seven cases with an embryonic heartbeat and six without. Laparoscopy and pathology confirmed the diagnosis of an ectopic pregnancy. Trophoblasts were identified in all 13 specimens by cytokeratin immunohistochemistry. Positive staining for cytokeratin, which stains all trophoblasts, is observed in Figure 3A and B. As a further example of implantation in an inappropriate environment, we studied a placenta accreta. We observed extravillous trophoblasts positive for HLA-G expression Figure 3D and E at the implantation site directly overlying the myometrium without intervening decidua. Thus HLA-G protein is also expressed in extravillous trophoblasts at an ectopic site. We conclude that HLA-G expression is most likely an intrinsic property of the differentiation of extravillous trophoblasts and is not dependent on appropriate environmental cues determined by interaction with the decidua. Since ectopic placentae are either accreta where the villous tissue has grown into the tubal musculature without intervening decidua or percreta where the villi penetrate and rupture the Fallopian tube we used a placenta accreta as a control and found that extravillous trophoblasts in the placenta accreta expressed HLA-G. The extravillous trophoblasts in ectopic pregnancies, unlike in a normal pregnancy, invade the Fallopian tubes and not the decidua. The extravillous trophoblasts in a placenta accreta also encounter an abnormal environment because the trophoblasts enter the myometrium without underlying decidua. Thus HLA-G expression correlates with the invasive trophoblast phenotype whether or not the normal tissue is invaded. The cellular environment encountered by extravillous trophoblasts in an ectopic pregnancy differs from that of the normal placenta in that there is no decidua. Benirschke and Kaufman reviewed the literature in which the placenta of ectopic pregnancies was discussed. They concluded that any reports of decidual cells in ectopic pregnancies are most likely mistaken and that the reported decidual cells are almost certainly X cells extravillous or intermediate trophoblasts. The extravillous trophoblasts in an ectopic pregnancy invade extrauterine tissue. The precise cellâ€”cell interaction at the fetalâ€”maternal interface is therefore lacking in ectopic pregnancies. However, it is possible that extravillous trophoblasts encountering any maternal tissue may induce expression of HLA-G which would also be the case in an ectopic pregnancy. Our conclusion that HLA-G is expressed without invasion into the decidua agrees with the observation that HLA-G is found in reverse transcriptionâ€”polymerase chain reaction RTâ€”PCR analysis of preimplantation embryos developed in vitro Jurisicova et al. This determination would be necessary to form a supporting conclusion from the preimplantation embryo studies. Furthermore, the preimplantation study was done on embryos developed in vitro which may not accurately depict HLA-G expression in vivo. One of the methods that developmental biologists use to study the effects of cell environment on cell differentiation is

transplantation of cells to an ectopic site in a living embryo reviewed in Gurdon et al. By studying extravillous trophoblasts in ectopic pregnancies we had the unusual opportunity of asking this basic question of developmental biology in the human. We conclude that induction of HLA-G expression in extravillous trophoblasts is most likely determined by autonomous regulation as opposed to its cellular environment. Clinical parameters of the ectopic pregnancy study group and HLA-G expression in their extravillous trophoblasts Case no.

Chapter 3 : HLA-G expression during preimplantation human embryo development. - Europe PMC Article -

We examined whether HLA-G is expressed during the critical period of preimplantation human development and whether expression of this molecule could be correlated with the cleavage rate of embryos.

Published online Mar The authors have declared that no competing interests exist. Conceived and designed the experiments: Participated in sample collection and embryo culture: Significantly contributed to manuscript revision: Received Nov 21; Accepted Feb This article has been cited by other articles in PMC. In the present study, we investigated the potential roles of HLA-G in human spermatogenesis and early embryonic development. There was no significant difference in HLA-G mRNA expression between testicular tissues with Johnsen score of 8â€”9 and normal sperm from ejaculated semen. In testicular tissues where sperm was obtained by testicular sperm extraction Johnsen score was 8 to 9 , there were no correlations between HLA-G mRNA expression and fertilization, cleavage and high-quality embryo rates. At 48â€”72 h post-fertilization, HLA-G expression was higher in fast growing embryos. Taken together, our findings suggested that HLA-G plays significant roles in human spermatogenesis and early embryonic development. HLA-G proteins can be expressed as seven distinct isoforms, by means of an alternative splicing from a single primary transcript. Membrane-bound HLA-G can be modified into soluble isoforms [1] , [2]. As an important immunomodulatory molecule, HLA-G inhibits cytotoxic activity of T cell, natural killer NK cell lysis and cell proliferation. HLA-G regulates the maturation, migration, transportation of dendritic cells and cross talk between T cell and NK cell. Except for its immune function, the non-immune function of HLA-G in reproduction has been discovered in recent years [2] , [7] , [8] , [9]. It is still controversial regarding the expression of HLA-G in precursor germ cells and spermatids. HLA-G has been detected in immune-privileged sites such as the placenta and the cornea [14]. Therefore, it is hypothesized that HLA-G not only expresses in male reproductive tissues, but also plays an important role in the regulation of male fertility. According to their observation, the expression of Mamu-AG mRNA in seminiferous tubules was strikingly cell specific and its expression was relatively weaker in germ cells and sertoli cells compared with that in spermatocytes. HLA-G5 protein was detectable in the cytoplasm of tubuloglandular epithelia and in glandular secretions, but not in prostate adenocarcinomas by immunohistochemistry [12]. In addition, positive signals of HLA-G were detected in normal testis and in epididymal tissue by immunohistochemistry staining [13]. Similar to the HLA-G expression in testicular tissues, inconsistent information existed regarding the HLA expression in oocytes and preimplantation embryos. Earlier research claimed that HLA type I and II were not expressed in human oocytes and preimplantation embryos [16] , [17]. However, Roberts et al. Results from more recent studies showed the percentage of HLA-G mRNA expression increased with the developmental stage of embryos [8] , [9] , [19]. In addition, the authors observed the overall pregnancy rate and the pregnancy rate after single embryo transfer were significantly improved when sHLA-G positive embryos were transferred. However, the underlying mechanisms remain largely unknown. Our results from this study demonstrated significant roles of HLA-G on both human spermatogenesis and embryonic development. We compared the HLA-G mRNA expression in testicular tissues with different spermatogenic ability, unfertilized oocytes and different stages of human embryos. Association of HLA-G expression in testicular tissues with Johnsen score of 8â€”9 with oocyte fertilization, embryo cleavage and high quality embryo rates were also investigated. Donated human gametes, embryos and testicular tissues were used with written informed consent received from patients seeking infertility treatment at the Center for Reproductive Medicine in the First Affiliated Hospital of Zhengzhou University from September to June Collection of testicular tissues, RNA extraction and reverse transcription RT Testicular sperm extraction TESE was performed prior to treatment with assisted reproduction, with part of the testicular tissues being sent to pathologic department for pathological analysis and the rest being frozen for future fertility treatment. Spermatogenesis was evaluated with the Johnsen scoring system [20] , where all tubular sections in each section of the testicular biopsy were evaluated

systematically and each was given a score from 1 to The criteria were as follows: A total of 46 testicular tissues were collected in the study, among which 7 were from patients with non-obstructive azoospermia Johnsen score 2â€”7 , 37 were from patients with obstructive azoospermia or hypospermatogenesis Johnsen score 8â€”9 , and 2 were from patients with difficulty in semen collection on the day of oocyte retrieval Johnsen score The concentration of extracted total RNA was measured by spectrophotometry. Sperm preparation and RNA extraction A total of 9 normal semen samples were included in this study. These couples received assisted reproductive therapy exclusively due to female factors. After 30 min liquefaction at room temperature, semen analysis was performed for the assessment of total sperm count, sperm motility and morphology. The mean age of the male patients are The sperm pellet was then washed twice by suspension in G-IVF solution, after which the supernatant was discarded and the sperm pellet was subsequently suspended in fresh G-IVF solution. The supernatant containing progressively motile spermatozoa was used for IVF, with the residual being centrifuged to remove the supernatant for RNA extraction. The in vitro matured oocytes were cryopreserved and stored in liquid nitrogen until use. Due to the difficulty in obtaining normally fertilized embryos for research, clinical useless embryos, including three-pronuclear 3PN zygotes the day after oocyte insemination and poor-quality embryos at 72 h post-fertilization were collected and used in this study. To obtain embryos at different developmental stages, some 3PN zygotes were cryopreserved 16â€”18 h post-fertilization day 1 and the rest were cultured until 44â€”46 h day 2 or 67â€”69 h day 3 post-fertilization prior to cryopreservation. Blastocysts were frozen â€” h post-fertilization and stored until use. Zona-free oocytes, zygotes, embryos and blastocysts were washed several times with G-MOPS to remove the remaining zona pellucida, granulosa cells and spermatozoa. Microinjection and embryo culture After warming, the vitrified 3PN zygotes were cultured in G-1 medium for 2â€”3 h before intracytoplasmic siRNA microinjection as described by Homer et al. Embryonic development was evaluated 48 h and 72 h post-fertilization respectively. PCR was carried out with the following conditions: The melting curves for a number of PCR products are single-peaked. The experiments were repeated three times. Fertilization rate was calculated by dividing the number of zygotes by total number of oocytes receiving ICSI. Cleavage rate means the number of cleaved embryos 48 h post-fertilization divided by the total number of cultured zygotes. High-quality embryo rate was defined as the percentage of high-quality embryos 72 h post-fertilization. The ratios were analyzed by the Chi-square test. A P-value of less than 0. Results HLA-G expressions are different in testicular tissues with different spermatogenic abilities Hematoxylin and eosin HE staining of testicular tissues was shown in Fig. A total of 17 testicular tissues were selected from the male patients aged Except for testicular tissues from azoospermia patients, two testicular tissues retrieved from normospermic patients due to difficulty in semen collection on the day of oocyte retrieval were also examined for HLA-G mRNA expression Fig. Both patients showed normal sperm count and sperm motility and Johnsen score was indicated as As shown in Fig.

Chapter 4 : Expression and Potential Roles of HLA-G in Human Spermatogenesis and Early Embryonic De

To verify this finding in a physiologically relevant setting, HLA-G protein expression was investigated during preimplantation development. We demonstrated HLA-G protein expression in oocytes, cleavage stage embryos, and blastocysts, where we find it in trophectoderms but also in ICM cells.

This article has been cited by other articles in PMC. It is thought to play a role in protecting the fetus from the maternal immune response. Interestingly, the preimplantation embryo development Ped gene product Qa-2 is also a mouse MHC class Ib protein that affects cleavage and division of preimplantation mouse embryos and subsequent embryonic survival. Data from many human in vitro fertilization IVF clinics suggest that the mouse Ped phenomenon also exists in human because embryos fertilized at the same time have different cleavage rates and consequently different IVF outcomes. As HLA-G is expressed in human early embryos, it is highly regarded as the functional homologue of Qa Whether HLA-G expression is correlated with the cleavage rate of human embryos has great potential clinical value. Methods In this study, 45 human early abnormal fertilized embryos 3 PN from patients undergoing in vitro fertilization were used to test the effects of HLA-G knock-down via infection with adenovirus carrying its specific siRNA on the cleavage rate in a 2-day culture period. A p-value smaller than 0. Results Knocking-down HLA-G in human pre-implantation stage embryos resulted in a higher cell arrest rate and a slower cleavage rate. Conclusion The results from the present study suggested that HLA-G might play an important role in early human embryo development. HLA-G is also expressed in preimplantation human embryos 1 â€” 4. Embryos capable of secreting soluble HLA-G are associated with a higher implantation rate, ongoing gestation and live birth rates in in vitro fertilization IVF cycles 5 â€” 7. More importantly, HLA-G expressing embryos have an increased development rate 1. Human HLA-G sequence possesses the highest homologue to that of the mouse Q7 and preimplantation embryo development Ped gene encoding Qa-2 protein 8. HLA-G and Qa-2 proteins also have similar functions 2 , 8 â€” It is also reported that the mouse Ped gene regulates the cleavage and division and subsequent survival of preimplantation mouse embryos Qa-2 is expressed in mouse preimplantation embryos from 2-cell through the blastocyst stage 14 , Two phenotypes of the Ped gene, fast and slow, have been defined. Ped fast preimplantation embryos develop at a significantly faster rate, both in vivo and in vitro, compared with Ped slow embryos 13 , 16 , HLA-G is suggested to be the human functional homologue of the mouse Qa-2 protein 2 , 8 , 9. The Ped gene phenotype, fast and slow preimplantation embryo development, also exists in human. Data from many IVF clinics showed that embryos from pools of oocytes fertilized simultaneously were not necessarily synchronized in their development, suggesting that gene s similar to Ped exist in human which could influence human preimplantation embryo cleavage In addition, embryos that divide more rapidly are more likely to lead to a pregnancy. The cleavage rate of embryos at 48 h after fertilization is significantly correlated with sHLA antigens in the culture medium HLA-G can act as a signaling molecule to induce proliferation of resting T cells We hypothesized that HLA-G could affect early human embryonic development. NM,, was served as a basis for the design of the two complementary mer oligonucleotides. For a negative control, the oligo sequence was scrambled and arranged not to affect any known human genes Table 1. A multiplicity of infection MOI of was applied for both the mouse and human embryos.