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Adenovirus Genome in the Placenta: Association with Histological Chorioamnionitis and Preterm Birth

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4 1 **Adenovirus Genome in the Placenta: Association with Histological**
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6 2 **Chorioamnionitis and Preterm Birth**
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17 **Short title:** Adenovirus in the placenta and preterm birth

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3 21 Adenovirus is isolated frequently from the amniotic fluid and has been implicated in severe
4
5 22 neonatal infections. A case control study was carried out to examine the association of detection
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7 23 of adenovirus in placentas with preterm birth and histological chorioamnionitis. Placentas from
8
9 24 preterm and full term deliveries were collected prospectively. Preterm cases were divided into
10
11 25 three subgroups according to the gestational age. PCR was carried out on placental tissues for
12
13 26 the detection of adenovirus genome. Placentas were evaluated histologically for the presence of
14
15 27 chorioamnionitis. Chi-square and odds ratios (OR) were used to determine if detection of
16
17 28 adenovirus is associated with preterm birth and histological evidence of inflammation. Seventy-
18
19 29 one preterm and 122 full term placentas were studied. Adenovirus genome was detected in 29
20
21 30 (40.8%) of preterm cases and in 25 (20.5%) of the full term controls (OR =2.6; 95% CI, 1.4-5.1;
22
23 31 $P=0.002$). Detection of adenovirus in preterm placentas was significantly higher compared to full
24
25 32 term particularly in the lower gestational age. Detection of adenovirus in placenta followed the
26
27 33 seasonal variation of adenovirus infections. Thirty-seven preterm and 21 full term placentas were
28
29 34 also selected for paraffin inclusion and histological examination. Chorioamnionitis was present
30
31 35 more frequently in preterm adenovirus-positive placentas compared to preterm adenovirus-
32
33 36 negative placentas (75% vs 36%; $P=0.03$) as well as compared to term adenovirus-positive
34
35 37 placentas (75% vs 19%; $P=0.003$). This study demonstrates that adenovirus infection of the
36
37 38 placenta is associated strongly with histological chorioamnionitis and preterm birth.

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40 **Keywords:** adenovirus; histological chorioamnionitis; placenta; preterm birth; viral infection

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41 INTRODUCTION

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43 Since Knox and Hoerner [1950] first recorded an association between infection and preterm
44 birth, there has been extensive evidence associating inflammation and infection to spontaneous
45 preterm birth, especially when this occurs at less than 28 weeks of gestation [Goldenberg et al.,
46 2003; Iams, 2003, Steer, 2005; Romero et al., 2007]. Various bacterial and viral agents have been
47 linked to the pathogenesis of preterm birth. Bacteria recovered from the amniotic cavity following
48 preterm delivery include *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*,
49 *Mycoplasma hominis*, *Ureaplasma urealyticum*, group B *Streptococcus*, *Bacteroides* spp., and
50 *Trichomonas vaginalis* [Goldenberg et al., 2003; Romero et al., 2007]. Despite the fact that viral
51 infection during pregnancy may prove to be devastating, evidence based etiology on the role
52 which specific viral infections may play in preterm birth still remains rare. A particularly
53 interesting finding is that adenovirus, a double-stranded DNA virus associated commonly with
54 conjunctivitis and upper respiratory tract infections, is detected more frequently in amniotic fluid
55 samples in comparison to other viruses such as cytomegalovirus, herpes simplex virus,
56 enteroviruses and parvovirus [Van den Veyer et al., 1998; Baschat et al., 2003a]. Congenital
57 adenovirus infections have indeed been associated with a variety of adverse effects on the fetus
58 ranging from complications such as pleural effusion, echogenic liver foci to more severe
59 complications including myocarditis and central nervous system anomalies [Meyer et al., 1985;
60 Baschat et al., 2003b].

61 In several case reports intrauterine adenovirus infections in preterm and full term neonates
62 have been reported to result in a fatal outcome [Towbin et al., 1994; Montone et al., 1995;
63 Ranucci-Weiss et al., 1998]. Perinataly-acquired systemic adenovirus infections in neonates have
64 also been described [Abbondanzo et al., 1989; Matsuoka et al., 1990; Abzug et al., 1991; Pinto et
65 al., 1992; Rieger-Fackeldey et al., 2000]. Adenovirus has also been detected in tracheal aspirates
66 from preterm neonates, within the first week of life, thus suggesting a possible mother to neonate
67 vertical transmission [Couroukli et al., 2000; Prosch et al., 2002].

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68 Several adenovirus serotypes are known to exhibit tropism for the genital tract, causing
69 cervical infection in women and urethritis in men [Lavery et al., 1977; Swenson et al., 1995;
70 Arnebrg et al., 1997; Bradshaw et al., 2002; Bradshaw et al., 2006]. Finally the expression of
71 coxsackie–adenovirus receptor (CAR) in the villous trophoblast cells, which is a prerequisite for
72 adenoviral infection of the placenta, has been demonstrated recently [Koi et al., 2001a; Koi et al.,
73 2001b].

74 In spite of all these observations a definitive association between adenovirus placental
75 infection and an adverse reproductive outcome has not been established. The purpose of this
76 study was to investigate in a case-control study, the detection of adenovirus in placentas in
77 conjunction with preterm birth and histological evidence of inflammation.

79 MATERIALS AND METHODS

80 Study design

81 During a twelve month period (1 January 2005 – 31 December 2005) all placental specimens
82 from spontaneous preterm deliveries were collected prospectively and consecutively, at the time
83 of delivery at the Alexandra University Hospital, Athens, Greece. Inclusion criteria consisted of
84 women who carried singleton pregnancies and delivered with a preterm birth secondary to
85 premature rupture of membranes, premature labour, or cervical insufficiency. Pregnancies with
86 congenital abnormalities of the fetus were excluded. Preterm cases were divided in three
87 subgroups according to gestational age (subgroup A: early preterm \leq 29wks; subgroup B:
88 intermediate preterm=30-33 wks; subgroup C: late preterm=34-36 wks). Gestational age was
89 calculated by dates and by an ultrasound scan. For each preterm case, one or two full term
90 specimens (\geq 37 weeks gestational age) were also collected consecutively at the same
91 approximate day with preterm placentas ($<$ 37 wks gestational age). For both groups various
92 demographic characteristics, including maternal age, race, obstetric history, estimated gestational

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3 93 age at delivery and fetal sex, were collected by the primary investigator (ET). The study obtained
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5 94 ethics committee approval and informed consent by all patients.
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8 **Placenta samples**

9
10 96 A total of 193 placenta samples, including part of the chorionic plate and chorionic villi, were
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12 97 stored at -80°C for adenovirus genome detection. Before DNA extraction placenta samples were
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14 98 washed in order to remove maternal blood from the intervillous space. In 58 cases, the peripheral
15
16 99 membranes were also sampled, then fixed in a 10% buffer formalin dilution and embedded in
17
18 100 paraffin for pathological examination.
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20 **Placenta DNA extraction and polymerase chain reaction (PCR)**

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22 102 PCR was used for the detection of adenovirus genome in the placenta tissue. Placental DNA
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24 103 extraction and PCR analysis were blinded to the gestational age of the sample. DNA extraction on
25
26 104 both case and control tissues was carried out using the Wizard Genomic DNA Purification Kit
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28 105 (Promega Corporation, Madison, Wisconsin, USA) according to the protocol provided by the
29
30 106 manufacturer. In order to verify that adequate amounts of DNA were extracted from all samples,
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32 107 a control PCR amplification of the beta-actin gene was performed on all specimens. Adenovirus
33
34 108 genome detection were performed using primers (hexAA1885: 5'-GCC GCA GTG GTC TTA
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36 109 CAT GCA CAT C-3'; hexAA1913: 5'-CAG CAC GCC GCG GAT GTC AAA GT-3') and
37
38 110 cycling parameters as describe previously [Allard et al., 1990]. With this method DNA of almost
39
40 111 all human adenovirus types derived from a wide range of clinical samples can be detected. All
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42 112 specimens were tested both neat and diluted by 10⁻¹ in order to verify the absence of inhibition.
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44 113 Positive and negative controls were included in all PCR experiments and genetic material was
45
46 114 extracted twice from each sample. In order to confirm the specificity of PCR products, the
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48 115 expected 308-bp adenovirus DNA amplicons were purified by a Qiaex gel extraction kit (Qiagen,
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50 116 Chatsworth, California, USA) and used as templates for sequencing of both strands with the ABI
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52 117 PRISM 377 DNA sequence analyzer (Perkin Elmer, Applied Biosystems Division, Foster City,
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54 118 California, USA).
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119 **Pathological examination**

120 Thirty-seven preterm birth and 21 control placentas which contained part of the peripheral
121 membranes were collected for histological examination of chorioamnionitis. Samples from the
122 peripheral membranes stained with routine hematoxylin-eosin were blinded and evaluated by
123 light microscopy to diagnose histological chorioamnionitis. The presence of neutrophils in the
124 membranes (chorion and/or amnion) was used to define chorioamnionitis.

125 **Statistical analysis**

126 Variables entering the study were gestational age (preterm versus full term placentas),
127 maternal age, the presence of adenovirus genome (PCR positive versus PCR negative), seasonal
128 distribution and the presence of chorioamnionitis (positive versus negative). Differences between
129 subgroups were evaluated using chi-square test. A *P* value < 0.05 was considered as statistically
130 significant. Odds ratios (OR) and their respective 95% confidence intervals (CI) were calculated
131 in order to estimate the magnitude of association between adenovirus genome detection and
132 gestational age. Logistic regression was used to adjust for possible confounders, such as maternal
133 age, in the detection of adenovirus. A statistical analysis was carried out using the statistical
134 package STATA 8.0.

136 **RESULTS**

137 **Characteristics of samples**

138 During the study period 233 placenta samples were collected prospectively (98 preterm and
139 135 full term samples). All spontaneous preterm deliveries were included. From preterm placenta
140 samples 48 were classified as late preterm (subgroup A), 30 as intermediate preterm (subgroup B)
141 and 24 as very preterm (subgroup C). Of those, 28 late preterm, 3 intermediate preterm and 13
142 full term samples were excluded from the study because the inclusion criteria were not fulfilled,
143 or due to inadequate sampling or storage procedure. Thus, 193 placenta samples (71 preterm and
144 122 full term placentas) which met the criteria were evaluated. From preterm placenta samples 20

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3 145 were late preterm (subgroup A), 27 intermediate preterm (subgroup B) and 24 very preterm
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5 146 (subgroup C).

7 147 Mean maternal age at delivery was 29.7 ± 5.6 years. Mean maternal age at preterm deliveries
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9 148 was 29.2 ± 5.4 years, while in the full term controls was 30.8 ± 5.8 years ($P > 0.05$). The two
10
11 149 groups had similar demographic features and between preterm and full term cases there were no
12
13 150 statistically significant differences as far as maternal age at birth, previous fetal loss or therapeutic
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15 151 abortions, previous gestations, nationality of mothers and fetal sex ($P > 0.05$).

18 152 **Molecular assays**

20 153 Adenovirus genome was detected in 54 (28.0%) of the total samples. From the 71 placenta
21
22 154 tissues of preterm birth, 29 (40.8%) were found positive for adenovirus genome, whereas from
23
24 155 the 122 placenta tissues of full term controls only 25 (20.5%) samples were positive for
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26 156 adenovirus by PCR ($P = 0.002$; Table 1). The probability of detecting adenovirus genome in
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28 157 preterm cases was estimated to be 2.7 times higher compared to term controls (OR=2.7, 95% CI,
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30 158 1.4-5.1). A similar association appeared when the subgroups of very and intermediate preterm
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32 159 placentas were compared to full term controls ($P = 0.026$; OR = 2.8; 95% CI, 1.1-7.0 and $P = 0.026$;
33
34 160 OR = 2.7; 95% CI, 1.1-6.5, respectively; Table 1). In the subgroup of the late preterm however,
35
36 161 this association was no longer significant statistically ($P = 0.056$; OR=2.6, 95% CI= 0.9-7.0; Table
37
38 162 1). It is also noteworthy that adenovirus detection was equally distributed among the subgroups of
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40 163 preterm placentas (Table 1).

42 164 Sequencing results of all adenovirus PCR-positive cases showed that the amplified sequences
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44 165 corresponded to the expected adenovirus hexon genome. Comparative genetic variability revealed
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46 166 that the amplified hexon gene sequences had similarities ranging from 97.7 to 100%. This
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48 167 variability between sequences almost excluded contamination events and confirmed validity of
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50 168 the study. Alignments of the amplified adenoviral sequences have also shown that most of the
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52 169 sequences corresponded to human adenovirus types of species C (n=41), while smaller numbers
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54 170 of them corresponded to human adenovirus of species B (n=8) and species A (n=5). The
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3 171 distribution of the adenovirus species did not differ between cases and controls. Adenoviruses
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5 172 that belonged to other species were not amplified.
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8 173 Multivariate logistic regression used to adjust gestational age for maternal age, showed that
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10 174 maternal age of full term or preterm cases did not affect significantly the detection of adenovirus
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12 175 genome ($P=0.38$). Adenovirus infection, among both preterm birth cases and controls, was more
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14 176 frequently detected during the spring months (March to June) following the seasonal distribution
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16 177 of adenovirus respiratory infections (Figure 1).
17

18 178 **Histopathology**

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20 179 Table 2 presents results of histological chorioamnionitis among 37 preterm and 21 term
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22 180 placenta samples. A statistically significant difference in the presence of chorioamnionitis was
23
24 181 found between preterm and full term placentas (49% vs 19%; $P=0.025$). Within the group of
25
26 182 preterm placentas, histological chorioamnionitis was significantly more common among
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28 183 adenovirus PCR-positive samples than among adenovirus-negative samples (75% vs 36%;
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30 184 $P=0.026$). Also, within the total adenovirus PCR-positive placentas, chorioamnionitis was more
31
32 185 frequent in preterm compared to full term samples (75% vs 19%; $P=0.003$). However, among
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34 186 adenovirus PCR-negative placentas, the pathological findings did not differ significantly between
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36 187 preterm and full term samples (36% vs 20%; $P=0.488$).
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42 189 **DISCUSSION**

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44 190 In the present case-control matched study, the detection rate of adenovirus genome in tissues
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46 191 from preterm placentas was compared with healthy full term cases. Although previous studies
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48 192 indicated that adenovirus is related to adverse pregnancy outcomes [Van den Veyer et al., 1998],
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50 193 this is the first prospective cohort study that documents an association between the presence of
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52 194 adenovirus genome in the placenta and spontaneous preterm birth. Baschat et al. [2003] reported
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54 195 an association between adenovirus isolation from the amniotic fluid and the presence of fetal
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56 196 echogenic liver lesions or neural tube defects, suggesting that adenovirus can be a fetal pathogen
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3 197 not recognized previously. Severe adenovirus infection in preterm neonates has been documented
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5 198 in several case reports. Abbondanzo et al. [1989] described fatal adenovirus pneumonia in a
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7 199 preterm neonate born at 25 weeks gestation. In this study the autopsy findings revealed that
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9 200 alveolar and bronchiolar lining cells contain frequently intranuclear inclusions and an
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11 201 overwhelming adenovirus infection was confirmed by *in situ* DNA hybridization. There is also
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13 202 evidence that viral acquisition from the mother, perhaps via the genital tract, is an important mode
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15 203 of adenovirus transmission [Abzug et al., 1991]. Molecular studies revealed that adenovirus
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17 204 serotypes 19 and 37 have a tropism for the genital tract [Arnerg et al., 1997]. In addition
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19 205 adenovirus is recognised as a rare cause of cervical infection or urethritis in men [Swenson et al.,
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21 206 1995; Lavery et al., 1977; Bradshaw et al., 2002; Bradshaw et al., 2006]. Couroucli et al. [2000]
22
23 207 isolated adenovirus from the tracheal aspirates of preterm neonates within the first week of life
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25 208 suggesting a possible vertical transmission from the mother to the neonate. Despite all the
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27 209 previous evidence, the role of adenovirus in preterm birth has not been investigated substantially.
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31 In the present study, preterm placentas were almost three times more likely to host adenovirus
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33 211 genome. Stratification by gestational age to early, intermediate and late preterm revealed that the
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35 212 earlier the gestational age the stronger the extent of this association, whereas in late preterm cases
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37 213 the association between adenovirus detection and preterm birth was no longer significant. These
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39 214 results are in line with the consensus that the role of infection in the etiopathogenesis of preterm
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41 215 labor pertains to the lower gestational age [Goldenberg et al., 2003; Iams, 2003]. However, it
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43 216 should be pointed out that with the primer sequences that were used some exotic types of
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45 217 adenovirus might have been missed from either cases or controls. It is of note that the PCR
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47 218 protocol in the present study does not use degenerated primers that are able to amplify all
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49 219 adenovirus genotypes [Allard et al., 2001]. It should be also mentioned that some of our full term
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51 220 and preterm samples were excluded from the study mainly due to inadequate sampling or storage
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53 221 procedure. Since the exclusion of these samples was random and not related with the study group
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55 222 we believe that the elimination of these specimens did not influence our results.
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3 223 The histological findings of the present survey showed that chorioamnionitis is associated with
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5 224 premature birth, as expected. This association remained significant in the subgroup of adenovirus
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7 225 PCR-positive samples, whereas it was no longer significant in the subgroup of adenovirus PCR-
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9 226 negative placentas. These findings reveal a meaningful association between adenovirus infection
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11 227 and histological chorioamnionitis, suggesting an etiological relation. Seropositivity data which
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13 228 would confirm this relation were not available in the present study. In a recent report
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15 229 [Arechavaleta-Velasco et al., 2008] adenovirus IgM antibodies were found more frequently in the
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17 230 serum of 24 women, who delivered preterm and sampled before 20 weeks of gestation. However,
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19 231 this study group was rather small and the sampling period was restricted for any safe conclusions
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21 232 to be drawn regarding the possible etiological role of adenovirus in preterm birth.
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25 233 The finding of increased adenovirus detection between March and June, following the
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27 234 seasonal variation in respiratory adenovirus infections, supports previous similar observations in
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29 235 samples from amniotic fluid and tracheal aspirates of preterm neonates [Prosch et al., 2002;
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31 236 Baschat et al., 2003a]. In conclusion, this study documents an association between adenovirus
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33 237 insult and preterm labour, possibly by causing chorioamnionitis expanding the spectrum of
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35 238 infectious pathogens which are related traditionally to chorioamnionitis. Further studies are
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37 239 warranted to investigate the pathogenic mechanisms relating adenovirus infection during
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39 240 pregnancy with placental inflammation and premature birth.
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44 242 No conflict of interest for all authors.
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REFERENCES

- 244
- 245 Abbondanzo SL, English CK, Kagan E, McPherson RA. 1989. Fatal adenovirus pneumonia in a
246 newborn identified by electron microscopy and in situ hybridization. *Arch Pathol Lab Med* 113:
247 1349-1353.
- 248 Abzug MJ, Levin MJ. 1991. Neonatal adenovirus infection: four patients and review of the
249 literature. *Pediatrics* 87:890-896.
- 250 Allard A, Albinsson B, Wadell G. 2001. Rapid typing of human adenoviruses by a general PCR
251 combined with restriction endonuclease analysis. *J Clin Microbiol* 39:498-505.
- 252 Allard A, Girones R, Juto P, Wadell G. 1990. Polymerase chain reaction for detection of
253 adenoviruses in stool samples. *J Clin Microbiol* 28:2659-2667.
- 254 Arechavaleta-Velasco F, Gomez L, Ma Y, Zhao J, McGrath CM, Sammel MD, Nelson DB, Parry
255 S. 2008. Adverse reproductive outcomes in urban women with adeno-associated virus-2
256 infections in early pregnancy. *Hum Reprod* 23: 29-36.
- 257 Arnberg N, Mei Y, Wadell G. 1997. Fiber genes of adenoviruses with tropism for the eye and the
258 genital tract. *Virology* 227:239-244.
- 259 Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP. 2003a. Prevalence of viral DNA in
260 amniotic fluid of low-risk pregnancies in the second trimester. *J Matern Fetal Neonatal Med*
261 13:381-384.
- 262 Baschat AA, Towbin J, Bowles NE, Harman CR, Weiner CP. 2003b. Is adenovirus a fetal
263 pathogen? *Am J Obstet Gynecol* 189:758-763.
- 264 Bradshaw CS, Denham IM, Fairley CK. 2002. Characteristics of adenovirus associated urethritis.
265 *Sex Transm Infect* 78:445-447.
- 266 Bradshaw CS, Tabrizi SN, Read TR, Garland SM, Hopkins CA, Moss LM, Fairley CK. 2006.
267 Etiologies of nongonococcal urethritis: bacteria, viruses, and the association with orogenital
268 exposure. *J Infect Dis* 193:336-345.

- 1
2
3 269 Couroucli XI, Welty SE, Ramsay PL, Wearden ME, Fuentes-Garcia FJ, Ni J, Jacobs TN, Towbin
4
5 270 JA, Bowles NE. 2000. Detection of microorganisms in the tracheal aspirates of preterm infants
6
7 271 by polymerase chain reaction: association of adenovirus infection with bronchopulmonary
8
9 272 dysplasia. *Pediatr Res* 47:225-232.
- 10 273 Goldenberg RL, Culhane JF. 2003. Infection as a cause of preterm birth. *Clin Perinatol* 30:677-
11
12 274 700.
- 13 275 Iams JD. 2003. The epidemiology of preterm birth. *Clin Perinatol* 30:651-664.
- 14
15 276 Knox IC, Hoerner JK. 1950. The role of infection in premature rupture of the membranes. *Am J*
16
17 277 *Obstet Gynecol* 59:190-194.
- 18 278 Koi H, Zhang J, Makrigiannakis A, Getsios S, MacCalman CD, Kopf GS, Strauss JF 3rd, Parry S.
19
20 279 2001a. Differential expression of the coxsackievirus and adenovirus receptor regulates adenovirus
21
22 280 infection of the placenta. *Biol Reprod* 64:1001-1009.
- 23 281 Koi H, Zhang J, Parry S. 2001b. The mechanisms of placental viral infection. *Ann N Y Acad Sci*
24
25 282 943:148-156.
- 26
27 283 Laverty CR, Russell P, Black J, Kappagoda N, Booth N. 1977. Adenovirus infection of the
28
29 284 cervix. *Acta Cytol* 21:114-117.
- 30
31 285 Matsuoka T, Naito T, Kubota Y, Morita Y, Takei J, Akiyama K, Hata J, Hasegava A, Sata T,
32
33 286 Kurata T. 1990. Disseminated adenovirus (type 19) infection in a neonate. Rapid detection of
34
35 287 the infection by immunofluorescence. *Acta Paediatr Scand* 79:568-571.
- 36
37 288 Meyer K, Girgis N, McGravey V. 1985. Adenovirus associated with congenital pleural effusion. *J*
38
39 289 *Pediatr* 107:433-435.
- 40
41 290 Montone KT, Furth EE, Pietra GG, Gupta PK. 1995. Neonatal adenovirus infection: a case report
42
43 291 with in situ hybridization confirmation of ascending intrauterine infection. *Diagn Cytopathol*
44
45 292 12:341-344.
- 46
47 293 Pinto A, Beck R, Jadavji T. 1992. Fatal neonatal pneumonia caused by adenovirus type 35.
48
49 294 Report of one case and review of the literature. *Arch Pathol Lab Med* 116:95-99.
- 50
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2
3 295 Prosch S, Lienicke U, Priemer C, Flunker G, Seidel WF, Kruger DH, Wauer RR. 2002. Human
4
5 296 adenovirus and human cytomegalovirus infections in preterm newborns: no association with
6
7 297 bronchopulmonary dysplasia. *Pediatr Res* 52:219-224.
8
9
10 298 Ranucci-Weiss D, UerpaiojkitB, Bowles N, Towbin JA, Chan L. 1998. Intrauterine adenovirus
11
12 299 infection associated with fetal non-immune hydrops. *Prenat Diagn* 18:182-185.
13
14 300 Rieger-Fackeldey E, Aumeier S, Genzel-Boroviczeny O. 2000. Disseminated adenovirus
15
16 301 infection in two premature infants. *Infection* 28:237-239.
17
18 302 Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. 2007. The role of
19
20 303 inflammation and infection in preterm birth. *Semin Reprod Med* 25:21-39.
21
22 304 Steer P. 2005. The epidemiology of preterm labor--a global perspective. *J Perinat Med* 33:273-
23
24 305 276.
25
26
27 306 Swenson PD, Lowens MS, Celum CL, Hierholzer JC. 1995. Adenovirus types 2, 8, and 37
28
29 307 associated with genital infections in patients attending a sexually transmitted disease clinic. *J*
30
31 308 *Clin Microbiol* 33:2728-2731.
32
33
34 309 Towbin JA, Griffin LD, Martin AB, Nelson S, Siu B, Ayres NA, Demmler G, Moise KJ Jr,
35
36 310 Zhang YH. 1994. Intrauterine adenoviral myocarditis presenting as nonimmune hydrops fetalis:
37
38 311 diagnosis by polymerase chain reaction. *Pediatr Infect Dis J* 13:144-150.
39
40 312 Van Den Veyver IB, Bowles N, Carpenter RJ Jr, Weiner CP, Yankowitz J, Moise KJ Jr,
41
42 313 Henderson J, Towbin JA. 1998. Detection of intrauterine viral infection using the polymerase
43
44 314 chain reaction. *Mol Genet Metab* 63:85-95.
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315 Table 1. Adenovirus genome detection in all preterm and in preterm subgroups according to
 316 gestational age vs full term controls.

Gestational age (wks)	PCR-positive <i>n</i> (%)	PCR-negative <i>n</i> (%)	Total	<i>P</i>	Odds ratio	95% CI
Term \geq 37	25 (20.5 %)	97 (79.5 %)	122			
Preterm < 37	29 (40.8 %)	42 (59.2 %)	71	0.002	2.7	1.4-5.1
Subgroup A: 34-36	8 (40.0%)	12 (60.0%)	20	0.056	2.6	0.9-7
Subgroup B: 30-33	11 (40.7%)	16 (59.3%)	27	0.026	2.7	1.1-6.5
Subgroup C: 29-22	10 (41.7%)	14 (58.3%)	24	0.026	2.8	1.1-7

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349 Table 2. Occurrence of histological chorioamnionitis in placenta tissues among different study
 350 groups.

	Histological choriamnionitis			χ^2 (<i>P</i> value)
	Positive <i>n</i> (%)	Negative <i>n</i> (%)	Total <i>n</i>	
Overall sample (<i>n</i> =58)				
Preterm	18 (49%)	19 (51%)	37 (64%)	
Full term	4 (19%)	17 (81%)	21 (36%)	4.99 (0.025)
Adenovirus PCR-positive group (<i>n</i> =28)				
Preterm	9 (75%)	3 (25%)	12 (43%)	
Full term	3 (19%)	13 (81%)	16 (57%)	8.86 (0.003)
Adenovirus PCR-negative group (<i>n</i> =30)				
Preterm	9 (36%)	16 (64%)	25 (83%)	
Full term	1 (20%)	4 (80%)	5 (17%)	0.48 (0.488)
Preterm group (<i>n</i> =37)				
Adenovirus PCR-positive	9 (75%)	3 (25%)	12 (32%)	
Adenovirus PCR-negative	9 (36%)	16 (64%)	25 (68%)	4.94 (0.026)

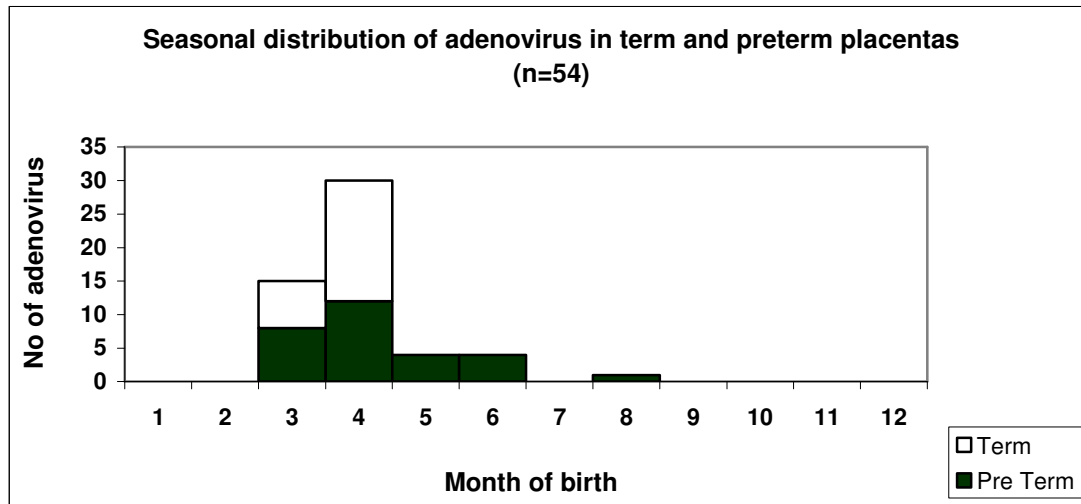
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354 **FIGURE LEGENDS**

355 **Fig 1.** Bar graph depicting the seasonal distribution of adenovirus genome detection in preterm
356 and term placentas.



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