

## Heterochromatin Variants in Slovak Women with Reproductive Failure

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**ABSTRACT** Various studies have reported a higher incidence of heterochromatin variants among individuals with idiopathic reproductive failure. The aim of the present study was to assess the frequency of chromosomal heteromorphisms in 948 women with history of reproductive failure and 478 controls in the Prešov region (Slovakia) (1998-2013) using G-banding and C-banding cytogenetic techniques. In 95 individuals (10.02%) with reproductive failure heterochromatin variants of chromosomes 1, 9, 16 and Y were detected. In the control group, there were 15 (3.15%) heterochromatin variants. The most frequent heterochromatin variants in the reproductive failure group were heterochromatin variants within chromosome 9 (9qh+/9qh-/inv(9)). The overall incidence of heterochromatin variants in women with reproductive failure was higher than in controls ( $p < 0.0001$ ; 95% CI 1.971-5.996). The results of the study confirmed the higher occurrence of chromosome anomalies in Slovak women with reproductive failure that absolutely reasons indication of cytogenetic examination.

### INTRODUCTION

Reproductive failure is defined as abnormal reproductive outcomes, such as infertility, recurrent spontaneous abortion or stillbirth and malformed childbearing history. Chromosome anomalies belong to genetic factors, which participate on etiology of reproductive failure. Human infertility is closely linked to chromosomal abnormalities (Šípek et al., 2014, Minocherhomji et al., 2009, Madon et al., 2005). Chromosomal heteromorphisms, known as chromosomal polymorphisms, include varying sizes of heterochromatin blocks, satellites, repeat sequence regions and inversions (Christofolini et al. 2012). Common cytogenetic polymorphisms detected by G-banding are considered as heteromorphisms and include heterochromatin regions of chromosomes 1, 9, 16 and Y and also prominent acrocentric short arms, satellites and stalks (Brothman, 2006). The most common inversion variant is inv(9)(p12q13) (Schaffer et al., 2012). The contradiction existed as to the consequences of the heterochromatin variations seen in the general population with respect to its inheritance. Cyto-

genetic studies are essential for evaluating of chromosomal aberrations and heterochromatin variants in women with reproductive failure. Karyotyping of the subjects with reproductive failure is important not only from a diagnostic new point, but even more importantly, to gain the better understanding of gametogenic impairment, which is associated with chromosomal abnormalities (Yassen et al. 2001).

### METHODOLOGY

The aim of the study was to determine the frequency of heterochromatin variants in the survey of women with reproductive failure ( $n=948$ ) and fertile control women ( $n=478$ ) in the Prešov region in Slovakia over a period of years 1998-2013. The study objects were Slovak women with reproductive failure with the history of primary and secondary infertility and repeated spontaneous/missed abortions cases (mean  $34.05 \pm 6.02$  years) with no explanations recruited by clinicians. Control group consisted of fertile women with children (mean  $31.07 \pm 7.13$  years). For cytogenetic analysis, peripheral blood samples were prepared according to the standard laboratory protocol (Rooney and Czepulkowski 1992). The cultures were incubated for 72 hours at  $37^\circ\text{C}$  in RPMI-1640 medium. The cell divisions were arrested in the metaphases by adding colchicine  $4 \times 10^{-5}\text{M}$  for 30-40 minutes before harvesting the cultures. The cultures were treated with 0,4% potassium chloride hypotonic solu-

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tion and fixed in 3:1 methanol-acetic acid mixtures. The banded slides were microscopically analyzed using G-banding and C-banding methods according to ISCN nomenclature (Mitelman 1995). Statistical analysis was performed with SPSS® version 17.0 statistical package (SPSS Inc., Chicago, IL, USA) for Windows®. Fisher's exact test was used to compute P-values and 95% confidence intervals (CI) for the odds ratios (OR). A P-value <0.05 was considered statistically significant.

## RESULTS

Cytogenetic analyses of 948 karyotypes of Slovak women with reproductive failure revealed chromosomal aberrations in 13 women with failed reproductive histories (1.37%). Chromosome aberrations detected in analysed survey of women with reproductive failure included numerical (0.53%) and structural (0.84%) chromosomal aberrations (Table 1).

In the survey of Slovak women with reproductive failure, there were 95 heterochromatin variants of chromosomes 1, 9, 16 (10.02%). In the control group, there were 15 heterochromatin variants (3.15%). The results are summarized in Table 2.

The most frequent heterochromatin variants in the reproductive failure group were heterochromatin variants within chromosome 9 (9qh+/9qh-/inv(9)). Heterochromatin variants of chromosome 9 and variant 13p+ were the common variants in the control group.

Statistically significant differences in the incidence of chromosomes 1, 9 and 15 heterochro-

**Table 1: Chromosomal findings in Slovak women with reproductive failure in the Prešov region (1998-2013)**

Chromosomal findings	Women (n = 948)
<i>Chromosomal aberrations</i>	13/948 (1.37%)
Numerical aberrations	47,XXX/46,XX [3] 45,X/46,XX [2]
Structural aberrations	45,XX,t(13;14)(q10;q10) 46,XX,t(1;15)(p34;q15) 46,XX,t(17;19)(q12;p13)
inversions	inv(4)(p13;q31), inv(9)(p13;q13) inv(3)(p11;q13)
mosaicisms	46,XX/46,XX,t(7;14)(q22;q11)
others	46,XX,der(12)? 46,XX,der(13;14)(q10;q10)
<i>Chromosomal heteromorphism</i>	95/948(10,02%)

matine variants were detected ( $p = 0.048$ , 95% CI 1.047–19.714;  $p=0.0377$ , 95% CI 1.111–9.288;  $p=0.016$ , 95% CI 1.316–24.046). The overall incidence of heterochromatin variants in the survey of women with reproductive failure was significantly higher than in the controls ( $p<0.0001$ ; 95% CI 1.971–5.996). The results of the present study confirmed the significant correlations of heterochromatin variants in Slovak women with reproductive failure (Table 3).

## DISCUSSION

Etiology of reproductive failure in human is a complex problem influenced by a lot of factors.

**Table 2: The frequencies of heterochromatin variants in Slovak women with reproductive failure and controls**

Chromosome No.	Heterochromatin variant	Group			
		Reproductive failure women (n = 948)		Controls (n = 478)	
		n	%	n	%
1	1qh+	13	1.79	1	0.42
	1qh-	4		1	
9	9qh+	12	2.53	2	0.84
	9qh-	8		1	
	inv(9)	4		1	
16	16qh+	7	1.05	1	0.21
	16qh-	3		0	
13	13p+	9	0.95	4	0.84
14	14p+	5	0.53	1	0.21
15	15p+	21	2.22	2	0.42
21	21p+	5	0.53	1	0.21
22	22p+	4	0.42	0	0
Total	95	<b>10.02</b>	15	<b>3.15</b>	

**Table 3: Statistical significance of differences in incidence of heterochromatin variants in the study group compared with controls**

	<i>Chromosome</i>								<i>Total</i>
	<i>1</i>	<i>9</i>	<i>16</i>	<i>13</i>	<i>14</i>	<i>15</i>	<i>21</i>	<i>22</i>	
P	0.048*	0.0377*	0.1199	0.9964	0.6182	0.016*	0.6182	0.3511	<b>p&lt; 0.0001</b>
95% CI	1.047- 19.714	1.111- 9.288	0.6811- 41.724	0.3670- 3.892	0.310- 22.797	1.316- 24.046	0.3763- 118.99	0.4421- 29.322	<b>1.971- 5.996</b>

Values are p- value according to Fischer exact test (95% CI for the OR)

\*Statistically significance

In some women with reproductive failure genetic account may be conclusive. The data about the incidence of chromosome anomalies in the surveys of women with reproductive failure alleged by individual authors are influenced by many factors and were rather disparate. At present, the relationship between heterochromatin variants and reproductive failure is still controversial. Many of the authors have reported a significantly elevated incidence of heterochromatin variants in the individuals with idiopathic reproductive failure (Bhasin 2005). Many studies reported higher incidence of heterochromatin variants in infertile couples that may suggest some impact on reproductive failure (Madon et al. 2005; Hong et al. 2011; Minocherhomji et al. 2009; Nakamura et al. 2001; Yakin et al. 2005). However, no widely acceptable explanation for this association has been published. The results of Madon et al. (2005) study showed polymorphic variants in 28.82 percent of males and 17.19 percent of females attending an IVF clinic with primary infertility or repeated miscarriages. Šípek et al. (2014) found that the heterochromatin variants occurred more frequently in the reproductive failure group than in the controls, the most significant difference between the study and control groups was found for the 9qh+ variant. Minocherhomji et al. (2009) in the case-control study identified a highly statistically significant increase in the frequency of total chromosomal variants in infertile women (28.31% vs. 15.16%) and infertile men (58.68% vs. 32.55%) as epigenetic alterations associated with the infertility phenotype. Brothman et al. (2006) concluded that common cytogenetic variants were considered to be heteromorphisms without clinical significance. Dong et al. (2013) found that chromosomal heteromorphisms do not play a role in reproductive problems. The etiological mechanism explaining this phenomenon has not yet been described.

The heterochromatin regions apparently contain a considerable amount of repetitious DNA, the repetitious DNA of these heterochromatin regions is heterogeneous. Chromosomal variants are an expression of morphological variability chromosome-related changes in the amount of heterochromatin. It is believed that the presence of chromosomal variant increases the risk of the non-disjunction of chromosome segregation. Heterochromatin has a specific role and behaviour in the synapsis of human homologous chromosome. One of the typical and common heterochromatin variants inv(9) has been repeatedly mentioned as more common in women (Uehara et al. 1992; Yamada 1992). Interchromosomal effect, leading to abnormal meiotic cell division and the development of disomic gametes is the other mechanism proposed for the pathological influence of the pericentric inversion of chromosome 9 and other heterochromatin variants. Some previous reports on the mechanisms of reproductive failure couples with an inv (9) carrier suggested that the crossing over in an inversion loop during meiosis leads to an unbalanced genetic composition of each chromosome (Boue et al. 1975). Chromosome 9 shows a high degree of morphological variability. Heterochromatin variants of chromosome 9 (9qh+/9gh-/inv(9)) are common findings in routine cytogenetics, with a frequency of 8 percent, 1 percent and 1.5 percent, respectively (Starke et al. 2002). Clinical implications of break points of this area were not yet specified. The prevalence of chromosome 9 pericentric inversion in the general population varies depending to ethnicity. Many of the studies describe pericentric inversion of chromosome 9 in association with infertility, recurrent pregnancy loss (Davolos et al. 2000), mental retardation, schizophrenia, Walker-Walburg syndrome, psychiatric and neurological disorders as well as with predisposition

to cancer (Teo et al. 1995). D/G-genome chromosomes heteromorphisms show increased heterochromatin at the chromosome telomere, the short arm, and the nucleolar organizing region (NOR). Chromatin variation in these regions causes defects in centromere function and kinetochore assembly, difficulty in homologous chromosome pairing, and impacts on cell division, thus, affects gamete formation. Heterochromatin in chromosomal polymorphism variations can regulate gene expression by reversible transformation between heterochromatin (non-coding DNA sequences) and euchromatin (expressed DNA sequences) (Frenster et al. 1973; Nakatsu et al. 1974). The clinical importance of heterochromatin variants is still not clear. Heterochromatin variants are inherited and are useful as genetic markers in linkage studies.

The results of the study confirmed the correlation of heterochromatin variants with reproductive failure. This findings reasons indication of cytogenetic examination. Heterochromatin variants probably are not the single determining factor for reproductive failure. Futher, the studies based on more detailed identification and description of heterochromatin variants by molecular cytogenetic methods are needed to explain the possible relation between heterochromatin variants and reproductive failure.

### CONCLUSION

The frequency of heterochromatine variants detected in the study support the opinion that heterochromatin variants may be asociated with reproductive failure. The results of the study indicated that relatively considerable part of Slovak women with reproductive failure have pathological cytogenetic findings, their significance need futher detailed studies. All these findings support genetic screening of individuals with reproductive failure.

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