

Alcohol-related Developmental Origin of Adult Health – population studies in Poland among mothers and newborns (2010-2012)

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Abstract

Alcohol related harm is a global problem for public health where frequent consumption of large amounts of alcohol constitutes a serious health risk, particularly to vulnerable groups such as adolescents, pregnant women and newborns. The epidemiological study on health-lifestyle behaviour, especially alcohol consumption, was performed on a randomised group of post-partum women's health behaviour during pregnancy, covering drinking habits, was undertaken in 2010, 2011 and 2012, (n=8,237) according to the PRAMS model including effects on the foetus and newborn; women being selected from obstetric and gynaecological wards. In this Polish study, only 14% of women did not consume alcohol before becoming pregnant while 15% of women drank alcohol throughout the entire period of pregnancy. In addition, awareness of the harmful effects of alcohol consumed, especially of small amounts, before and during pregnancy is low among Polish women. It is also alarming that more than 55% of physicians who provide care for pregnant women do not discuss with them the harmful effect of alcohol on the organism of the mother and foetus, whereas over 2% of doctors even recommend the consumption of alcohol in pregnancy. With reference to the Barker's Foetal Origin of Diseases Hypothesis, the authors suggest such alcohol drinking behaviour of women during their reproductive ages and while pregnant may exert negative health effects on offspring, mainly in the form of susceptibility to contracting chronic diseases. Such findings pose a risk to future generations in Poland and require remedial/educational action targeted on health care professionals and public like.

Key words

Barker's Foetal Origin of Diseases Hypothesis, epigenetics, foetal development, pregnancy outcome; alcohol

INTRODUCTION

The effects of drinking alcohol during pregnancy were first documented in the scientific literature in 1973 in an article in the *Lancet*. Here, the Foetal Alcohol Syndrome (FAS) was first identified and defined, the signs and symptoms of which are the most severe and commonly seen after chronic alcohol consumption [1]. These symptoms primarily include pre-prenatal and/or postnatal growth retardation disorders, consisting of deficiencies in weight gain, height and head circumference, [2] which appear in later years, even when the environment is optimal for child development [3]. Studies have demonstrated reduced levels of growth factors in offspring [4, 5, 6] resulting in decreased cell proliferation, particularly in the brain [7], and influencing its reduced mass. It should also be pointed out that differences in gene expression of those enzymes regulating alcohol metabolism will obviously govern the predisposition of an individual to the harmful

effects of alcohol [8, 9]. Craniofacial abnormalities are also seen in FAS, consisting of small eye openings with inner epicanthic folds present, a sunken and weakly formed nasal bridge, short palpebral fissures, smooth philtrum and thin vermilion border of the upper lip and large ears. A cleft palate may also occur as can a small jaw [10]. In 40% FAS cases, limb abnormalities are seen and often deformations of the ribcage, and underdevelopment of the urethral muscles and genitalia [11, 12, 13, 14]. Cardiac defects are observed in 29-50% of FAS cases, most frequently of the cardiac septal type [15, 16]. For a long time, alcohol withdrawal symptoms, (i.e. delirium tremens), have been recognised in those newborns whose mothers drank alcohol during pregnancy following which the infants had problems suckling and breast feeding [12, 17]. They were also restless, had weakly coordinated sight with movement and were frequently subject to epileptic attacks [18]; all signs of a damaged cerebellum. FAS is recognised as being one of the main causes of mental retardation [19, 20, 21], as demonstrated by an average IQ of 65 in children with FAS.

Alcohol consumption during pregnancy may not always present the whole clinical range of FAS symptoms. In 1996, a committee to study FAS (authorised by the USA Congress),

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from the American Institute of Medicine (IOM), published a report with recommendations [22] on diagnosis, prevention epidemiology and treatment of alcohol-pregnancy related disorders. Inherited defects and developmental disorders resulting from alcohol exposure were classified into 4 categories; the aforementioned FAS; Partial Foetal Alcohol Syndrome (P-FAS), where only certain symptoms arising from CNS (central nervous system), disorders appear together with some craniofacial abnormalities; Alcohol-Related Neurodevelopment Disorder (ARND), characterised chiefly by neurobehavioral disturbances [23] and; Alcohol-Related Birth Defects and Developmental Disorders (ARND), where mainly physical deformations are manifest. In 2004, a new term was introduced: 'Foetal Alcohol Spectrum Disorders' (FASD), that covers an entire range of physical, mental, behavioural and learning disabilities/disorders resulting from maternal alcohol drinking during pregnancy [2, 24, 25, 26], and which appear in different guises and in varying degrees. Furthermore, the effect of mothers drinking identical amounts of alcohol, and for the same durations in pregnancy, can vary between individual foetuses [27]. Behavioural problems and cognitive deficits (attention span, short-term memory), have, likewise, been shown to be affected by drinking during pregnancy, in childhood, and throughout adolescence [28, 29, 30, 31].

There is much evidence to show that intrauterine exposure to alcohol causes genetic changes resulting in inborn defects [32, 33] where differences in DNA sequence, (polymorphism), are seen in those genes responsible for metabolising alcohol [34, 35, 36]. The gene coding for alcohol dehydrogenase (ADH), has been most extensively studied and is responsible for converting alcohol to acetaldehyde [37]; the latter being mutagenic [38]. Indeed, acetaldehyde has been recognised by the IARC (International Agency for Research on Cancer), to be carcinogenic [39]. Other studies have identified genes with alcohol-related teratogenic potential and susceptibility to FASD [32, 40].

A very significant observation is that epigenetic changes occur following alcohol exposure. Epigenetics describes lasting and potentially reversible alterations to cellular genetic function (i.e. gene expression), which are non-DNA related, (i.e. not through mutation), and where these epigenetic changes at defined gene loci depend on receiving certain environmental triggers, (e.g. in-utero alcohol exposure). Such changes can be maintained long after the environmental signal has been removed and may thereby give rise to FASD [41]. The mechanism of epigenetic action is the regulation of gene expression at the level of transcription, i.e. RNA and protein synthesis, whereas modifications to DNA are responsible for determining and maintaining gene expression programmes in different cell types. Despite identical DNA sequences, differences in mammalian gene expression result in over 200 different cell types that constitute various tissues and organs, i.e. although mammalian tissue has the same genotype they differ by phenotype and thus function [23].

Epigenetic processes occur via DNA-chromatin complexes, histone proteins and other proteins making up the chromosome where epigenetic alteration occurs in both histones and DNA; a well-known example of the latter being DNA methylation. Here, the DNA's cytosine nucleotide, followed by a guanine and separated by a phosphate (CpG dinucleotide), becomes methylated. [42, 43]. This can occur in the gene promoter region, (governing the first stages of transcription), or in

other parts of the gene responsible for activating or repressing gene expression of histones. Modifications to histones are however chiefly through acetylation, phosphorylation and methylation, among others (Fig. 1).

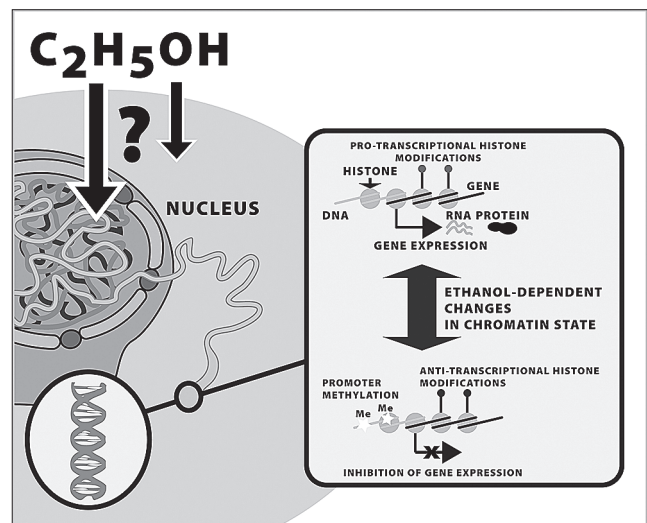


Figure 1. Epigenetic effect of alcohol on gene expression.

Follow: Kobar MS Weinberg J, Focus on: Epigenetics and Fetal Alcohol Spectrum Disorders Alcohol Research & Health, Vol. 34, No. 1; <http://pubs.niaaa.nih.gov/publications/arh341/29-37.htm>

The state of chromatin can be altered by environmental factors. Chromatin is composed of DNA segments coiled around histones and situated in the cell nucleus. This DNA packaging system is dynamic and its local state regulates gene expression through transcription to ultimately synthesise protein. DNA methylation of particular segments, i.e. cytosine nucleotides in the regulatory gene promoter region, inhibits transcription of this gene. Histones can also become modified by the substitution of various other molecules which may either inhibit transcription (i.e. are antitranscriptional), or conversely can be protranscriptional, depending on the nature of group substitutions, the surrounding environment and state of chromatin. Both DNA methylation and histone modification are dynamic processes under multi-enzymatic regulation, the activities which are influenced by many environmental factors, such as substrate availability in methylation. Ethanol, for instance, can disrupt the homocysteine-methionine cycle by reducing levels of S-Adenosylmethionine which acts as a methyl-group donor for DNA or histone methylation [23] (Fig.1).

The relationship between alcohol and the epigenome was discovered through many human and animal studies. For example, alcohol disrupts the homocysteine-methionine cycle in many places [44, 45, 46]. Methionine is an essential amino acid which, by definition, can only be obtained from the diet and is vital for correct cellular function, including protein synthesis. It is the precursor for S-Adenosylmethionine (SAM), whose generation from methionine, in the aforementioned cycle, leads to homocysteine synthesis. Methionine itself is regenerated by folic acid dependent/independent homocysteine methylation. SAM acts as the main methyl group donor in the DNA and histone methylation, and alcohol disrupts this SAM dependent methylation by acting on the enzymes of the homocysteine-methionine cycle [47, 48, 49]. Disruption of DNA methylation is also a key part of the aetiology of alcohol related liver disease [50, 51].

Both alcohol and its metabolites (chiefly acetaldehyde), cause specific modification of histones as demonstrated in cultured rat liver cells treated with alcohol [51, 52]. Alcohol exposure of hepatic cells alters gene expression in a stable fashion, and thus may constitute the epigenetic memory. It also reduces folic acid uptake, a key element in the homocysteine–methionine cycle by which means methylation is affected [53, 54]. Abnormal methylation can be observed in the promoter regions of those white blood cell genes that are active at various stages of development in chronic alcoholics [55, 56]. The role of epigenetic mechanisms, as a result of prenatal exposure to alcohol, is now universally accepted [43], and indeed the earliest evidence for this was from rat studies as long ago as 1913. This showed a greatly increased offspring mortality when the father rats had been previously exposed to alcohol; an effect carried onto the next generation. Many subsequent studies have confirmed this, where the offspring of rat parents that had been exposed to alcohol before conception had decreased body weight and height, and resulted in smaller litter sizes and more inborn abnormalities [23, 57, 58, 59, 60]. It has been suggested that the alcohol action is mediated by reducing gene expression of the enzyme methylating DNA (i.e. DNA methyltransferase1 – DNMT1), as shown in the substantially decreased levels of this enzyme in the sperm of rats exposed to alcohol for 9 weeks [61]. Similar findings have been observed in human volunteers at sites where hyper-methylation was normally expected to occur but, in fact, DNA methylation was much reduced [62]. The transmission of epigenetic alterations to the offspring after fertilisation affects gene expression and abnormal prenatal development. Decreased infant body weight is observed when the mother drinks alcohol at about the time of conception [63, 64, 65]. This has been confirmed by studies on male mice who had free access to alcohol before conception, and whose offspring showed epigenetic changes in a specific gene allele [66] and other studies demonstrating disruption of gene expression after long-term alcohol exposure, also in male mice [64]. The preimplantation period (between fertilisation and uterine implantation), which in mice lasts 4-6 days post-fertilisation and 2 weeks in humans is also the time when zygote development is sensitive to epigenetic alteration. Changes in the mouse foetus become apparent when exposed to alcohol and result in growth retardation and physical deformation on the 15th day of the pregnancy [67], which has also been recently confirmed in humans [68]. Furthermore, the placenta and embryo exposed to alcohol through maternal consumption show growth retardation compared to a control group. Genetic imprinting has been observed to play a large role in this delay of development before the occurrence of embryonic implantation [43].

Gastrulation is an early stage in embryonic development, starting at implantation. In mice, this starts and finishes at 6 and 14 days, respectively, after conception. In humans, this lasts between 3 and 6 weeks of the pregnancy. During this time organ development starts where there is a dynamic cellular differentiation. This period is recognised as being the most sensitive time for the teratogenic effects of alcohol to occur [23]. Since the early 1990s, alcohol-related epigenetic alterations have been shown to occur during gastrulation [69] where female mice given alcohol between 9-11 days of pregnancy caused hypo-methylation of foetal DNA; most likely, as mentioned previously, through alcohol inhibiting DNA methyltransferase –DNMT. Because changes in

DNA methylation may result in altered gene expression, it is considered that alcohol changes the gene expression programme and can give rise to developmental disorders of FASD [23, 64].

These findings have been confirmed in studies where reaction to stress was evaluated in 5-7-month-old human fetuses whose mothers had limited their alcohol intake once they became aware of their pregnancy. They demonstrated an increased heart rate and arousal, together with elevated cortisol, a stress hormone, aside from its other functions [70]. These observations imply there are underlying epigenetic mechanisms behind such foetal behaviour. Alcohol also affects the foetus in its later stages of intrauterine development, as seen by alcohol exposure in the 3rd trimester, resulting in memory disorders and information processing indicative of damage to the hippocampus [71, 72]. Alcohol, in fact, is more toxic for women than men arising from the smaller water content of the female body and a lowered capability of alcohol clearance as a consequence of diminished liver enzyme activity which metabolise this substance [73]. Research demonstrates that consuming 60g of neat alcohol per week is enough to increase the risk of stillbirth by 2-3 times and miscarriage by 4 times [74]. During pregnancy any alcohol consumed by the mother readily passes through the placental barrier to the foetus. Here, alcohol exposure is all the greater as foetal metabolism and clearance is much lower compared with that of the mother [75, 76]. When ethanol and its main metabolite, acetaldehyde, reach the amniotic fluid their elimination is also very slow. Ethanol causes free radical formation that damages cellular protein and lipids and it negatively affects cell division and differentiation into various types of cells and tissue. Retinoic acid synthesis is also limited; this being a substance regulating embryogenesis. Consuming 20g of ethanol in one go is sufficient to elicit foetal breathing movement disorders and retard other movements. Alcohol metabolism depends on the presence of cytosolic alcohol dehydrogenase in hepatocytes. Foetal levels of this enzyme's activity reach about 50% of adult levels, moreover, it is entirely absent in the foetal brain and placenta; clearly then alcohol clearance is much lower than in the mother [77]. At around the 16th week of pregnancy, the foetus begins to express the Cytochrome CYP2E1 enzyme that oxidises ethanol; after 24 weeks, 10-30% of adult levels are achieved, whereas full activity is only attained after 10 years of life [78]. Summing up, alcohol readily passes the placental barrier and the foetus has limited capacity for its clearance, thereby it has a low alcohol tolerance [79].

Recent and extensive reviews have shown that even small amounts of alcohol consumed during pregnancy may cause abnormal CNS development which, in later years can result in behavioural and cognitive dysfunction [80, 81, 82, 83]. It has been hypothesised that the epigenetic changes due to environmental influences (including alcohol consumption), on the embryo and foetus shape the cellular and tissue phenotype of the F1 generation, as well as those of the F2. This demonstrates that maternal alcohol consumption affects the foetal phenotype and that of the child, as well as the child's germ cells, i.e. on the F2 phenotype [84, 85, 86, 87].

Consuming alcohol during pregnancy exposes 3 generations to its harmful consequences through inherited epigenetic alterations; in the first place, the mother is directly affected, then the foetus, followed by the foetal germ cells [88] (Fig. 2).

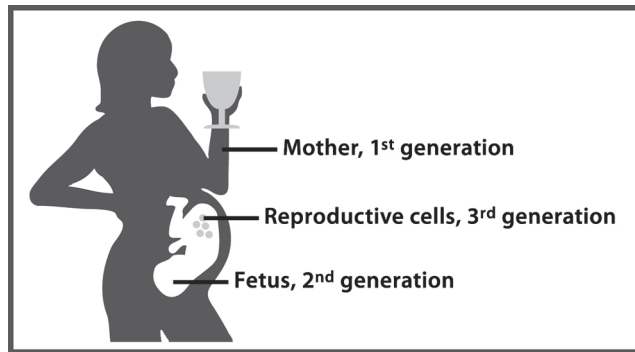


Figure 2. Consumption of alcohol by a woman in pregnancy has negative health effects on the mother and foetus, and causes epigenetic changes in reproductive cells of the foetus.

Follow: Perera F, Herbstman J. Prenatal environmental exposures, epigenetics, and disease. *Reprod Toxicol.* 2011;31 (3): 363-73. (modified)

RESEARCH HYPOTHESIS

It is conjectured that in Poland, alcohol consumption in youth during the reproductive period, and by pregnant women may have harmful consequences on the pregnancy, childbirth, newborn status and infant health.

METHODOLOGY

In Poland, a population survey was conducted during 2009-2012 in hospitals among women in childbirth,

(Questionnaire, Part 1), together with the staff providing the healthcare which included doctors and nurses in neonatal units, (Questionnaire, Part 2). In 2009, the survey was carried out in a face-to-face manner, by trained staff from health education departments of the District Sanitary-Epidemiological (SANEPID). Studies conducted in 2009 were of a pilot character and not analyzed in the presented report. From 2010 onwards, the questionnaires were filled out by the subjects themselves. In 2010-2011, the questions were expanded to all concerned and this format was retained for 2011-2012. Healthcare staff, (gynaecologists and nurses), provided answers according to official medical documentation, i.e. using maternal and newborn care cards covering the pregnancy, childbirth and time spent in hospital after the birth. Answers were entered into an appropriate data base at the Lublin Institute of Rural Health. In each of the 4 study years, the survey was conducted in different months, i.e. June 2009, August 2010, November 2011 and March 2012, to account for any seasonal variations. The diagram below shows the methodology of the Pregnancy-related Assessment Monitoring Survey (PRAMS).

STATISTICAL ANALYSIS

The study was performed using the Statistica 8.1 PL computer package. Variables were presented as frequency tables, descriptive statistics and contingency tables. In the latter, the relationships between categorical variables were analysed by the Pearson Chi² test.

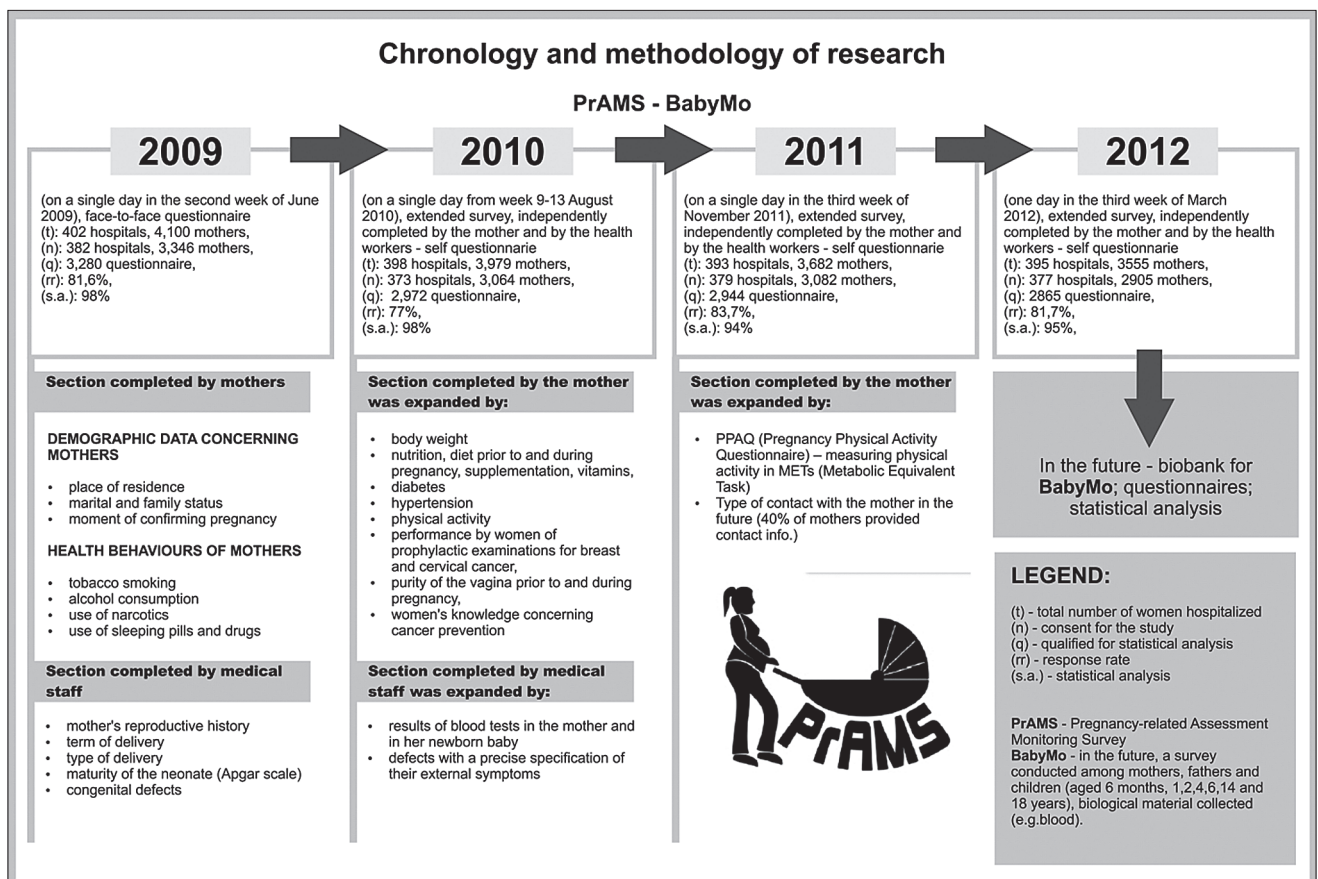


Figure 3. Chronology and methodology of PrAMS research in Poland

RESULTS

Statistical analysis covered 8,237 women from all of Poland who, after delivery, were hospitalized together with their newborn babies, and correctly completed questionnaire forms. More than 50% (50.31%) of Polish women in childbirth were aged 26-32 and more than 57%, (57.46%), were urban inhabitants while 42.5% lived in the rural areas. A statistically higher percentage of rural women in younger age groups gave birth (under 22-16.78% in the rural areas and 10.40% in urban areas). One third of urban women gave birth aged 29-32 compared to 23% of rural women (Tab. 1).

Table 1. Characteristics of the population examined

		2010						
		<=22	23-25	26-28	29-32	33-35	>=36	Total
Urban area	n	158	240	356	462	185	124	1,525
	%	10.36	15.74	23.34	30.30	12.13	8.13	57.57
Rural area	n	177	203	262	288	112	82	1,124
	%	15.75	18.06	23.31	25.62	9.96	7.30	42.43
Total	n	335	443	618	750	297	206	2,649
	%	12.65	16.72	23.33	28.31	11.21	7.78	32.16
		2011						
		<=22	23-25	26-28	29-32	33-35	>=36	Total
Urban area	n	193	206	384	440	198	162	1,583
	%	12.19	13.01	24.26	27.80	12.51	10.23	56.17
Rural area	n	228	204	292	261	124	126	1,235
	%	18.46	16.52	23.64	21.13	10.04	10.20	43.83
Total	N	421	410	676	701	322	288	2,818
	%	14.94	14.55	23.99	24.88	11.43	10.22	34.21
		2012						
		<=22	23-25	26-28	29-32	33-35	>=36	Total
Urban area	n	141	179	347	519	245	194	1,625
	%	8.68	11.02	21.35	31.94	15.08	11.94	58.66
Rural area	n	183	195	263	270	121	113	1,145
	%	15.98	17.03	22.97	23.58	10.57	9.87	41.34
Total	n	324	374	610	789	366	307	2,770
	%	11.70	13.50	22.02	28.48	13.21	11.08	33.63
		TOTAL						
		<=22	23-25	26-28	29-32	33-35	>=36	Total
Urban area	n	492	625	1,087	1,421	628	480	4,733
	%	10.40	13.21	22.97	30.02	13.27	10.14	57.46
Rural area	n	588	602	817	819	357	321	3,504
	%	16.78	17.18	23.32	23.37	10.19	9.16	42.54
Total	n	1,080	1,227	1,904	2,240	985	801	8,237
	%	13.11	14.90	23.12	27.19	11.96	9.72	100.00

More than a half of women in the survey (54.75%), before becoming pregnant, consumed alcohol once a month or more rarely, while 7.4% of respondents declared that they consumed alcohol several times a week, including 5.63% – 2-3 times a week, and 1.77% – at least 4 times a week (Tab. 2). An alarming observation is that women aged up to 22 years consumed the most alcohol ie. at least 4 times

Table 2. Frequency of alcohol consumption by women before becoming pregnant and during pregnancy (sample data combined from 2010 + 2011 + 2012), according to age categories

		Consumption of alcohol before pregnancy (p chi ² =0.076)					
		4 x and more/week	2-3x/week	2-4x/month	1x and more rarely/month	not at all	Total
<=22	n	13	22	102	254	67	458
	%	2.84	4.80	22.27	55.46	14.63	12.51
23-25	n	7	32	130	313	84	566
	%	1.24	5.65	22.97	55.30	14.84	15.46
26-28	n	9	51	200	496	117	873
	%	1.03	5.84	22.91	56.82	13.40	23.84
29-32	n	26	63	273	524	148	1,034
	%	2.51	6.09	26.40	50.68	14.31	28.24
33-35	n	8	27	96	225	56	412
	%	1.94	6.55	23.30	54.61	13.59	11.25
>=36	n	2	11	65	193	48	319
	%	0.63	3.45	20.38	60.50	15.05	8.71
Total	n	65	206	866	2,005	520	3,662
	%	1.77	5.63	23.65	54.75	14.20	100.00
		Consumption of alcohol during pregnancy (p chi ² =0.552)					
		4 x and more/week	2-3x/week	2-4x/month	1x and more rarely/month	Not at all	Total
<=22	n	4	3	9	64	378	458
	%	0.87	0.66	1.97	13.97	82.53	12.51
23-25	n	2	2	7	84	471	566
	%	0.35	0.35	1.24	14.84	83.22	15.46
26-28	n	1	1	9	114	748	873
	%	0.11	0.11	1.03	13.06	85.68	23.84
29-32	n	5	1	14	127	887	1,034
	%	0.48	0.10	1.35	12.28	85.78	28.24
33-35	n	5	2	6	52	347	412
	%	1.21	0.49	1.46	12.62	84.22	11.25
>=36	n	2	1	6	40	270	319
	%	0.63	0.31	1.88	12.54	84.64	8.71
Total	n	19	10	51	481	3,101	3,662
	%	0.52	0.27	1.39	13.13	84.68	100.00

weekly. After becoming pregnant 84.68% of women totally abstained from drinking alcohol in any form whatsoever. However, as many as 13.13% women sporadically consumed alcohol when pregnant (once a month, or more rarely). Considerable numbers of women regularly consumed alcohol in pregnancy: 2-4 times a month (0.27%) or more often (0.79%). Women aged 22 and under, admitted having the highest alcohol consumption in pregnancy; 0.87% of them consumed alcohol at least 4 times a week, 0.66% – 2-3 times a week, 1.97% – 2-4 times a month, and as many as 13.97% once a month or more rarely (Tab. 2). The trend towards an increasingly earlier alcohol initiation is clearly observed among adolescents, as well as an increasingly more common acceptance and, consequently a lack of social awareness concerning the negative consequences of alcohol consumption by pregnant women. It is an alarming fact that after becoming pregnant, a considerable percentage of women who declared that before pregnancy they consumed

alcohol 4 times a week, or more often (29.23%), and those who consumed alcohol once a month, or more rarely (23.99%), did not discontinue this consumption, nor even reduce their alcohol intake.

A considerably higher consumption of alcohol in the preconception period was noted among urban than rural women. A very frequent consumption of alcohol (2 times a week or more rarely) was mentioned by 9.05% of urban and 4.75% of rural women (Tab. 3). It is disturbing to find that, respectively, 27% and 6.19% of women, living in rural and urban areas, made no attempts to either stop or reduce their alcohol consumption. Occasional alcohol consumption in pregnancy was declared by more than 13% of women, irrespective of the place of residence. Total abstinence during pregnancy was reported by 84.70% of urban women and 84.58% of rural women (Tab. 3).

Table 3. Frequency of alcohol consumption by women in the preconception period and during pregnancy (sample data combined from (2010 + 2011 + 2012), according to the place of residence

Consumption of alcohol before pregnancy (p chi ² <0.000)							
		4 x and more/week	2-3x/week	2-4x/month	1x and more rarely/month	not at all	Total
Urban area	n	45	165	619	1,207	285	2,321
	%	1.94	7.11	26.67	52.00	12.28	62.58
Rural area	n	20	46	260	814	248	1,388
	%	1.44	3.31	18.73	58.65	17.87	37.42
Consumption of alcohol during pregnancy (p chi ² =0.085)							
		4 x and more/week	2-3x/week	2-4x/month	1x and more rarely/month	not at all	Total
Urban area	n	9	4	38	304	1,966	2,321
	%	0.39	0.17	1.64	13.10	84.70	62.58
Rural area	n	11	7	14	182	1,174	1,388
	%	0.79	0.50	1.01	13.11	84.58	37.42

As many as 39.77% (n=3,430) of the total number of respondents confirmed that they consumed alcohol or energizing beverages during pregnancy; the majority drinking red wine, (n=1,465), or beer, (n=1,265). Significant numbers of pregnant women also admitted to drinking high alcohol spirits, (n=194), or wine fortified with high proof alcohol, (n=34). More than 15% of respondents declared that they consumed low alcohol beer when pregnant. More than 2% of the women examined provided a positive answer to the question concerning the consumption of energizing drinks/coolers (Tab. 4).

Consumption at one time of the mean amount of alcohol, expressed in so-called alcohol units (330ml beer 4.5%= 14.85 ml/11.9g ethyl alcohol; 175ml wine 12%=21ml/16.8g alcohol; 50ml vodka 40%=20 ml/16g alcohol) by women who mentioned alcohol consumption during pregnancy, only slightly decreased and was: 2.147±2.170 before pregnancy and 1.560±1.989 during pregnancy.

As many as 822 respondents admitted that they aimed to become inebriated (consumption of > 3 alcohol units at one time), and among this number, 7 achieved this state also during pregnancy (Tab. 5). At the same time, a considerable number of women (n=189) who had a tendency to become inebriated admitted a consumption of 1-3 alcohol units at one time during pregnancy.

Table 4. Characteristics of alcohol/energizing beverages consumed by pregnant women (3,430 respondents out of the total number, n=8,237*)

	n	% of replies	% of cases
low alcohol beer	645	15.81	18.80
beer	1,265	31.00	36.88
red wine	1,465	35.91	42.71
white wine	394	9.66	11.49
fortified wine	34	0.83	0.99
coolers	83	2.03	2.42
High alcohol content spirits	194	4.75	5.66
Total	4,080	100.00	118.95

* possibility to select more than one answer.

Table 5. Occurrence of women being inebriated, (consumption of > 3 alcohol units at one time*) by the women in the study before and during pregnancy (822 from among the total number of n=8,237 respondents provided a positive answer)

		<=3 units	>3 units	Total	p chi ²
before pregnancy	n	749	73	822	0.013
	%	91.12	8.88	100.00	
during pregnancy	n	189	7	196	
	%	96.43	3.57	100.00	

* alcohol unit:
330 ml beer 4.5% = 14.85 ml/11.9 g ethyl alcohol
175 ml wine 12% = 21 ml/16.8 g ethyl alcohol
50 ml vodka 40% = 20 ml/16 g ethyl alcohol

More than a half of the women in the survey (55.01%) admitted that the physician in charge of their pregnancy did not discuss with them the problem of alcohol consumption during pregnancy. In the case of 3,330 women, the physician recommended to abstain from any amount of alcohol during pregnancy (Tab. 4). A considerable number of women were informed by the physician that the consumption of small amounts of alcohol is allowable/recommended in order to maintain the state of pregnancy (n=71), or for other, not mentioned reasons (n=98).

Table 6. Recommendations/contraindications provided by the physician providing care during pregnancy associated with alcohol consumption by pregnant women (7,777 from among the total number of respondents n=8,237, provided an answer)

	n	%
Physician did not discuss the problem of alcohol consumption in pregnancy	4,278	55.01
Physician recommended total abstinence from alcohol during pregnancy	3,330	42.82
Drinking small amounts of alcohol allowed by physician for maintenance of pregnancy	71	0.91
Drinking small amounts of alcohol allowed by physician for reasons other than maintenance of pregnancy	98	1.26
Total	7,777	100.00

Table 7 shows wide variations in the respondents' awareness of the potential risks/health threats of drinking alcohol, as well as other risky lifestyle behaviour, to the foetus or mother during pregnancy. Nearly all the women admitted that

the consumption of high amounts of alcohol created a high risk for the health and life of mother (96.42%) and foetus (98.22%). Nevertheless, awareness of negative consequences of the consumption of small amounts of alcohol in pregnancy was considerably lower: 45.97% and 61.25%, respectively – which was the percentage of women who were aware of the risks for the mother and the foetus related with the consumption of small amounts of alcohol during pregnancy. Nevertheless, the fact that some respondents (n=184) do not in any way regard that drinking small amounts of alcohol is harmful to foetus or mother while pregnant, is a cause for concern. Active and passive tobacco smoking was considered by nearly 90% of women in the study as a high or moderate risk factor, both for the mother and the developing foetus. According to 88.78% of respondents, active smoking by pregnant women constitutes a high risk factor for the developing foetus. Furthermore, pregnant women in a smoking environment was considered a high risk situation to both the developing foetus, (79.09%), and to the mother's health, (67.34%) by those surveyed. Nearly all respondents were aware that the use of narcotics is a high risk factor for the health and life of the mother (98.61%) and the foetus (99.21%). Awareness of the harm caused by taking psychoactive substances (the so-called designer drugs/legal highs), other than narcotics was also at similar levels (Tab. 7).

Table 7. An evaluation of the pregnant women's graded risk awareness to the potential harm caused by selected risky lifestyle behaviour to foetal/maternal health and life; results combined from 2010, 2011 and 2012

Self-reported assessment of risk/threat for health and life of the mother							
		small amounts of alcohol	high amounts of alcohol	active smoking	passive smoking	narcotics	other psychoactive substances
Lack of risk	n	400	34	66	75	37	18
	%	5.01	0.42	0.83	0.95	0.46	0.34
Slight risk	n	1,319	44	277	450	17	20
	%	16.52	0.55	3.49	5.70	0.21	0.38
Moderate risk	n	2,595	209	1,135	2,055	57	136
	%	32.50	2.61	14.29	26.02	0.72	2.58
High risk	n	3,670	7,729	6,464	5,319	7,851	5,101
	%	45.97	96.42	81.39	67.34	98.61	96.70
Self-reported assessment of risk/threat for health and life of the foetus							
		small amounts of alcohol	high amounts of alcohol	active smoking	passive smoking	narcotics	other psychoactive substances
Lack of risk	n	184	26	36	38	27	13
	%	2.31	0.32	0.45	0.48	0.34	0.25
Slight risk	n	874	11	159	261	8	11
	%	10.95	0.14	1.98	3.28	0.10	0.21
Moderate risk	n	2,035	106	704	1,366	28	77
	%	25.50	1.32	8.79	17.16	0.35	1.45
High risk	n	4,888	7,888	7,113	6,297	7,959	5,197
	%	61.25	98.22	88.78	79.09	99.21	98.09

These findings very clearly demonstrate that women drink alcohol at around the time of conception which then affects the pregnancy, type of delivery and status of the newborn. Furthermore, in accordance with the 'Developmental

Origins of Adult Health and Disease' model, this behaviour affects the health of the offspring in later life, as well as that of the subsequent generation. This hypothesis was confirmed by the presented study.

DISCUSSION

Worldwide, drinking alcohol during pregnancy is still rife, despite the many efforts made by public health authorities to forestall this harmful behaviour; thus constituting a global problem [89]. Moreover, there are large differences between countries [90]. The USA and Canada show similar numbers of pregnant women drinking, about 5-15% [91, 92, 93], however, in many European countries and Australia this number is greater [94]. In France, only 52% women abstained from alcohol consumption during pregnancy, and in Australia 81% drank alcohol [95, 96]. Data from Italy indicate that 30% of mothers giving birth drink alcohol daily and that becoming aware of being pregnant is not a reason for limiting alcohol consumption [97]. Around 16% of South Korean mothers drink alcohol at all stages of pregnancy and 1.7% actually become inebriated [98]. A Danish study has shown that 50% of pregnant women become inebriated at least once during the time their menstrual cycle was conducive for conception [99]; a similar result was observed in Ireland [100]. In the presented study only 12% of urban women and 17% of rural women did not consume alcohol before becoming pregnant. Approximately 15% of pregnant Polish women consume alcohol throughout the entire period of pregnancy. This is consistent with other Polish studies [101, 102]. It seems that frequent drinking of large amounts of alcohol is higher among the youngest age groups. Drinking alcohol before conception and the first 2 weeks critically affects growth and foetal development, as well as health and normal development in later years [103, 104]. In many East European states, a large increase in alcohol consumption has recently been noted, owing, it seems to the expanding advertising of alcohol in the mass media – this being related to the relatively recent democratic changes achieved in these countries. For example, in Russia, alcohol consumption has risen by 80% during 1990-2005 [105]. Longitudinal studies conducted in Moscow also showed that 85% of pregnant women drank alcohol of which 20.2% became binge-drunk at least once during pregnancy [106]. Other Russian studies demonstrated even higher numbers of pregnant women who drank alcohol in this way, ranging from 26%-60% [107, 108]. There was no evidence of Russian women lowering their alcohol intake when planning to become pregnant; only when pregnancies were medically confirmed did the frequency of drinking decrease [109]. In the USA, around 50% of pregnancies are unplanned, and women generally become aware of this 4-6 weeks after conceiving, i.e. at the end of the 1st trimester [110, 111]. Until that time, they drank the same as before being pregnant. Therefore, the previously described toxic effects of alcohol on the foetus are particularly harmful during this critical phase of development [112, 113]. Further USA studies show that women actually admitted that they continued to drink as before, until their pregnancy was medically confirmed [114]. In addition, 52.4% of women in the USA admitted to drinking alcohol in the last month of pregnancy, of which 11.5% also admitted to binge-drinking. In response, a report with recommendations was issued

and widely disseminated by the US Public Health authorities on preventing alcohol abuse in pregnancy, together with information on the harm thus caused [115]. A federal advisory committee was also set up in 2002, 'National Task Force on Foetal Alcohol Syndrome and Fetal Alcohol Effect' (NTFFASFAE), which issued the first recommendations on alcohol consumption during pregnancy [116]. Acting on this basis, the US Surgeon-General issued recommendations concerning the harm caused by drinking when pregnant as well as a strategy for tackling this habit in women during their reproductive years and in pregnancy [117]. Similar recommendations are necessary in Poland.

The first diagnosis of FAS was actually made in 2004 [118]. Since then, it has been estimated that in the USA and some other countries, the FAS incidence varies between 0.2-2 cases per 1,000 childbirths [119, 120, 121]. This is consistent with the results of the presented study. In 2009, part of our survey had certain questions addressed to medical healthcare staff on whether any FAS-like symptoms had occurred in newborns without direct reference to the actual FAS. Results showed that not a single answer confirmed FAS in the newborns. In subsequent years, however, the questionnaire included a list of externally obvious FAS symptoms, mainly concerning craniofacial deformation, and results then showed agreement with those observed abroad. It was thus concluded that healthcare staff in Poland are unfamiliar with FAS symptoms in newborns. In 1998, the US Congress passed legislation entitled 'The Alcoholic Beverage Warning Label Act' [122] which placed an obligation on the packaging of all alcoholic drinks so that product labels contained warnings that alcohol should be forbidden when pregnant, and that drinking while pregnant may cause defects in foetal development [123]. It was observed that after passing this Act, alcohol consumption in women did actually drop, especially among occasional drinkers [124, 125]. Thus, when taking the presented study results into account, similar regulations are required and necessary in Poland.

There are very many different methods extant for promoting alcohol abstinence while pregnant. However, in most countries, alcohol consumption in women of reproductive age has increased, and in parallel the numbers of unplanned pregnancies has also risen [103]. It is therefore important that alcohol prevention programmes, as an integral part of public health, are evidence based. The American 'Task Force on Community Preventive Services', supported by the CDC, has issued a special Community Guide with recommendations for the design, running and assessing of public health population promotion programmes and policy aimed at local government and NGOs [126]. These recommendations are scientifically based on literature reviews and address many of the health issues and threats dealt with at various organisational levels present within the structure of local authorities, NGOs and private organisations (e.g. workplace programmes or in private education). In addition to alcohol, other problem areas are focused on, such as tobacco smoking, lack of physical activity, and the so-called diseases of civilisation – diabetes and cancer. Since its inception in 1946, the WHO has always had alcohol as one of its priorities, and in 2003 the WHO 'Alcohol and Public Policy Group (APPG)' published recommendations on strategy and interventions, again based on scientific literature review and international experience [3]. The interventions were divided into 7 fields of action as follows: regulating alcohol availability, regulation

of prices and tax, changing drinking habits, education/raising awareness of the consequences of alcohol abuse, advertising and marketing, drink-driving, and treatment/early intervention. The most successful of the WHO strategies so far, in first place, have been on setting a minimum drinking age, government monopoly of alcohol, increasing prices, regulating the retail of alcohol, limiting when alcohol can be bought, (i.e. during a day/week), and intervening in those with alcohol addiction problems. In second place are measures such as increasing alcohol-free recreational activities, education about the harm caused by alcohol abuse in schools/universities, waging media campaigns and distributing warning posters. Currently, many wide-ranging strategies aimed at the general public are designed to limit alcohol at the time of conception, irrespective of the risk. These include public education on the harm caused by drinking, especially in women of reproductive age or whilst pregnant, informing about specific consequences, such as FAS and FASD [127], limiting alcohol availability and increasing excise duty [3]. This involves issuing educational materials, conducting media campaigns and appropriate labelling of alcoholic beverages [128]. Using public warning/educational posters and signs has proved effective in raising awareness on alcohol abuse, particularly in places where alcohol is sold/distributed [129, 130]. As an example, a recent warning poster campaign in New York raised awareness that alcohol causes inbred disorders by 14% [130]. Indeed, other studies [129] have also shown the effectiveness of alternative methods for propagating information concerning damage to the foetus due to drinking alcohol when pregnant. Warning signs/posters not only raise women's awareness, (during their reproductive age; 18-40 years), but actually lead to limiting their drinking whilst pregnant. Selective strategies and their implementation are used to target people/population groups that have been identified to be at greater risk from drinking, compared to the general public. This obviously includes the aforementioned women of reproductive age and those at pre-conception, who have a tendency to abuse alcohol [3]. Especially important is the role of the GP (General Practitioner).

Many medical professional organisations worldwide use an obligatory questionnaire test for detecting alcohol drinking problems in women of reproductive age [131, 132, 133, 134]. The WHO also recommends that this type of screening is performed using the 'Alcohol Use Disorder Identification Test (AUDIT-questionnaire)' [135, 136]. In the USA, which shows the greatest alcohol abuse in this group of women, other screening test-questionnaires are used by doctors in order to identify problem individuals; these include T-ACE, TWEAK and AUDIT-C, as well as CRAFFT, which is aimed at teenagers [131, 135, 137, 138, 139, 140]. Except for the latter, each test carries within its acronym key questions on which the tests are based on. For various reasons, however, only one third of doctors ever perform such specially designed tests [141, 142, 143, 144, 145, 146, 147] on this patient group, despite the recommendations of the US Surgeon General and other related professional organisations which make this type of testing mandatory [148]. According to the CDC, doctors experience difficulties in identifying women with alcohol problems in their reproductive years [149]. This is compounded because women decrease their drinking as soon as they become aware of being pregnant, and also because they consider that drinking small amounts during pregnancy

is acceptable [3, 150]. Despite these difficulties, such screening tests conducted by GPs are an effective means of detecting alcohol abuse in women of reproductive age or when pregnant [151, 152]. Poland is also a country where this group of women believe there is no harm in drinking small amounts of alcohol when conceiving/pregnant. What is also worse, is that Polish gynaecologists recommend that there is no harm in drinking a small amount of alcohol while pregnant; a very occasional glass of wine is acceptable for relaxing due to the often stressful nature of pregnancy. Furthermore, a significant number of Polish women will drink anyway while pregnant. In the presented studies more than 50% of physicians providing care for pregnant women (gynaecologists-obstetricians), did not discuss with them the harm caused by consuming alcohol when pregnant. It is an alarming fact that over 2% of physicians recommended alcohol consumption in pregnancy. It is therefore vital that recommendations are prepared and issued by both professional associations and the medical authorities for highlighting the harm caused by alcohol during pregnancy. It is also necessary to implement educational programmes on this issue aimed at raising awareness, especially for sexually mature youth, as well as ensuring that the message reaches women of reproductive age that alcohol harms the pregnancy, the foetus and the newborn offspring. As yet in Poland, there have been no recommendations prepared by any healthcare/medical organisations; whether they be family doctor associations nor obstetric and gynaecology societies. Government institutions have likewise not initiated any education or health promotion in this area; an important measure would be to educate students. The age at which students attend university/college makes it the most risky time for alcohol abuse [153, 154] and, as stated previously, most unplanned pregnancies are associated with drinking alcohol at the time of greatest foetal vulnerability [155, 156]. Despite this, alcohol consumption has recently risen in female students [157]. An interesting fact observed in the presented study is that slightly more than 45% of pregnant women were of the opinion that drinking small quantities of alcohol is unsafe for the mother's health, whereas 60% thought the same about the harm this would cause to the foetus. The awareness of the risk caused by tobacco smoking or taking psychactive substances in Poland is considerably higher than that for alcohol, where this awareness on the harm due to smoking tobacco during pregnancy is particularly high; indicative that educational campaigns targeted at this area have proved effective [158]. Interventions at this age are especially aimed at altering health lifestyle behaviour by learning how to cope with stress and dealing with the culture of alcohol drinking [154]. A widely adopted intervention is the 'Brief Alcohol Screening and Intervention for College Students' (BASICS) programme which comprises assessing individual exposure to the harm caused by alcohol and how this can be averted [159]. Just attending 2 sessions of this programme has been shown to be highly effective [160] as the young female students become aware of the threats posed by their alcohol drinking habits. Nevertheless, such action is not undertaken in Poland, despite the existence of a specialist 'Solving Alcohol Problems' agency.

Education is a key area in families where there is alcohol addiction as FASD occurs between generations. It is estimated that 13-25% of children from alcoholic parents, themselves become alcoholics [161, 162]. Latest literature reviews report

that interventions made in such circumstances are highly effective [162]. They consist of providing reliable information on the harm resulting from drinking alcohol when pregnant, and promoting health lifestyle and social behaviour which are a healthy alternative to drinking, such as sport. A part of the remit of the 'National Agency for Solving Alcohol Problems' does, in fact, identify the groups at risk; however, they are not engaged in disseminating knowledge on the harmful effects of alcohol during women's reproductive age, at pregnancy, nor on the foetus or offspring.

Conclusions based on the current state of knowledge:

- Alcohol consumption may be the cause of preterm deliveries and abortions.
- Consumption of alcohol increases the risk of developing alcohol-related defects in a newborn. The following disorders may occur in an infant: growth deficiency, impairment of the infant's central nervous system, and facial deformities. These abnormalities are commonly defined as Foetal Alcohol Syndrome (FAS). This syndrome is a part of many abnormalities associated with the undesirable effects of prenatal contact with alcohol, defined as Foetal Alcohol Spectrum Disorder (FASD), into which are additionally qualified behavioural and intellectual developmental disorders.
- Any amount of alcohol is dangerous for pregnancy and the foetus.
- Alcohol may damage the foetus at each stage of its development.
- Alcohol-induced foetal damage may occur as early as in the first weeks of pregnancy, before it is confirmed in the mother.
- Exposure of the foetus to alcohol results in the occurrence of cognitive and behavioural deficits at all later stages of life.

Due to the above-mentioned reasons:

- Pregnant women should not consume alcohol in any form.
- Women who consume alcohol prior to pregnancy should stop drinking in order to minimize its effects.
- Women who plan pregnancy should abstain from alcohol consumption.
- Health services staff should routinely ask pregnant women concerning their alcohol intake, inform them about the negative effects of alcohol on pregnancy and the foetus, and recommend that they abstain from alcohol consumption in pregnancy.

Recommendations:

- Professional medical organisations in Poland, particularly the College of Family Physicians and the Polish Gynaecological and Obstetrics Society, should prepare recommendations concerning the harm caused by drinking alcohol by women of reproductive age and those who are pregnant. These should include the negative effects of alcohol on pregnancy, the foetus, and offspring of future generations.
- Screening tests should be introduced in GP practice so that women, particularly the younger, with alcohol problems can be identified throughout their reproductive age.
- The harmful effects of even consuming small amounts of alcohol on the pregnancy and foetus should be taught to medical students and doctors undergoing post-graduate education.

- Government institutions, especially the National Agency for Solving Alcohol Problems, together with the Chief Sanitary Inspectorate, should introduce programmes that raise awareness of women at reproductive age, especially the younger ones, on the harm caused by alcohol consumption, not only during pregnancy but during their reproductive period, particularly after the age of 18.
- It is necessary for legislation to be introduced which makes it obligatory for alcoholic products to carry appropriate warning on their labels, stating the harm alcohol causes at pregnancy and for those women who plan to have a baby.

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