

Biophysical Properties Of Amniotic Fluid And Morphological Characteristics Of The Placenta In Primiparous And Multiparous Women Living At Different Altitudes In Kyrgyzstan

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ABSTRACT

Background & Purpose of the study: Infant mortality remains at a high level in Kyrgyzstan. The most unfavorable situation is in multiparous women living in highlands. The purpose of the work to determine the comparative characteristics of the surface-active properties of amniotic fluid and placental structure in nulliparous and multiparous women, who live in the lowlands, midlands and highlands. **Method:** The surface tension of amniotic fluid was measured and organometric, histologic, morphometric studies of 57 placentas were performed. **Results:** Macroscopic measurements showed that in nulliparous women weight, diameter and thickness of the placenta tend to increase with an increase in the height of residence. In multiparous women as compared to primiparous women the size of placenta decreases, insignificantly in lowlands and significantly at medium and high elevations. Also highlands multiparous showed a significant shortening of umbilical cord. Most primiparous women have a central attachment of the umbilical cord regardless of the altitude of residence. In multiparous women from the midlands and highlands there is a considerable frequency of the paracentral and marginal attachment. The microscopic structure of the placenta had specific characteristics in primiparous and multiparous women, which grew more intense with increasing altitude of residence. Pathologic villous immaturity in primiparous was observed in only one case (the highlands), in multiparous this condition was seen with a frequency of 10% in the lowlands to 42.8% in the highlands. In primiparas from highlands surface activity was significantly reduced. In multiparous women from lowlands SA levels of amniotic fluid did not differ significantly from those of primiparous women. In the midlands and highlands the shifts in SA were more pronounced in multiparous than primiparous women. Besides, a 33% decrease of the amniotic fluid SI in multiparas from high-mountain areas suggests immaturity of the lungs of newborns. **Conclusion:** Signs of placental insufficiency and subsequent development of respiratory distress syndrome in infants of multiparous women from highlands were revealed. .

Key-words: Placenta, Morphometry, Amniotic fluid, Surface tension, Fetoplacental insufficiency, High altitude, Multiparas.

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Infant mortality remains at a high level in Kyrgyzstan, higher than in Europe and the CIS countries.^{1,2} The most unfavorable situation is in the remote mountainous

regions.³ For the solution of this problem "National Strategy for Reproductive Health Protection of the Kyrgyz Republic until 2015" and "Program for Perinatal

Care Improvement in the Kyrgyz Republic for 2008-2017" were adopted. Besides organizational difficulties (shortage of doctors, problems with communication and transportation, repair of medical equipment, etc.), ethnic characteristics of the population and climatogeographic features of the terrain should be taken into consideration in regional health care planning. Families with many children have become a revered tradition of Kyrgyzstan.⁴ Multiparous women are those women who have given birth to 5 or more children. They give birth to their last children at the age of 35 years or more. In addition to this, a woman lives in difficult climatic and geographical conditions of high mountains, her physiological reserves are reduced by this time.⁵ In such a difficult pregnancy, changes occur in the entire system "mother-placenta-child" and morphological signs of placental insufficiency can be found.⁶ Babies are born with low birth weight and immature. In high altitude conditions, respiratory distress syndrome of newborn (RDS) can develop, which is based on immaturity of the lungs and, in particular, lack of lung surfactant.⁷ There are different ways to correct surfactant deficiency.^{8,9} For the prevention and early treatment of RDS it is important to develop a prediction system using the evaluation of amniotic fluid and after birth. Review of scientific literature indicates intensive studies in this field of research.^{10,11} The purpose of the study was to carry out a comparative study of the characteristics of the structure of the placenta in primiparous and multiparous women living in lowlands, midlands and the highlands.

MATERIALS AND METHODS

Amniotic fluid and placentas were collected in the Osh City Hospital (Osh, an altitude of 800 m above sea level), maternity wards of the Alai Territorial Hospital (village Gulcha, 1400 m) and the Chon-Alai District Hospital (village Darovat-Kurgan, 3000 m) from healthy women aged above 18 years and distributed into 6 representative groups: lowlands primiparas (n=10), midlands primiparas (n=10), highlands primiparas (n=10), lowlands multiparas (n=10), midlands multiparas (n=10), highlands multiparas (n=7). The average age of primiparas from the lowlands, midlands and highlands was $21,3 \pm 1,3$ years. The average age of multiparas was $33,2 \pm 1,4$ years without difference between the altitudes of residence.

The following methods were used:

1. Macroscopic examination of placenta (mass, volume, surface area of the uterine surface, length of umbilical cord, placental-fetal index - PFI).
2. Histological study of the placenta in paraffin sections stained with hematoxylin-eosin and by van Gieson method.
3. Computed morphometry of chorionic villi using the digital camera CAM V200 and BioVision software for medicine and biology with the determination of the diameter (length), and the volume density of villi.
4. Determination of the surface activity of amniotic fluid on tensiospectrometer SCI-001.

To do this, 10 ml of amniotic fluid was frozen in maternity homes and

delivered to the Department of Pathologic Morphology. Surface activity was determined on tensiospectrometer TCM-001 as described by Belov GV et al.¹² The volume of fluid to be analyzed was brought up to 100 ml with physiological saline, corresponding to the working volume of the cuvette of tensiospectrometer. Minimum surface tension (ST_{min}) and maximum surface tension (ST_{max}) were measured, which were used to calculate stability index (SI) by Clements formula:

$$SI = \frac{2(ST_{max} - ST_{min})}{(ST_{max} + ST_{min})} \quad (1)$$

RESULTS AND DISCUSSION

In primiparous women the mass, diameter and thickness of placenta tended to increase with increasing altitude of residence, although changes for the given sample (n=10) were not significant (see Table 1). At the same time, the placental-fetal index in primiparous women from highlands was significantly higher. In multiparous women as compared to primiparous women the size of placenta decreases, insignificantly in lowlands and significantly at medium and high elevations (p<0,05). Also highlands multiparous showed a significant shortening of umbilical cord.

Table 1 - Organometric parameters of placenta in primiparous and multiparous women

Parameters	Primiparous			Multiparous		
	LM	MM	HM	LM	MM	HM
Weight (g)	470,4±20,5	501,0±21,2	481,2±25,1	412,2±23,5	422±18,6	370,1*,** ±19,4
PFI	0,176±0,01	0,183±0,02	0,193 *±0,02	0,171±0,02	0,181±0,02	0,180 **±0,01
Diameter (cm)	19,3±0,7	19,6±0,9	19,5±0,9	18,1±0,7	17,3 **±0,8	14,7 *,**±0,8
Thickness (mm)	34,1±1,4	35,5±1,3	34,7±1,6	32,2±1,4	29,5 **±1,3	22,2 *,**±1,4
Length of umbilical cord (cm)	62,5±1,5	61,8±1,3	59,9±1,4	61,5±1,1	61,7±1,4	56,7±1,5

Note: * - difference compared to the low mountain group p<0.05

** - difference compared to the primiparous group p<0.05

A rounded shape of the placenta was revealed in most primiparous women from the lowlands, midlands and highlands - 90%, 80% and 70%, respectively (see Table 2). In multiparous women an irregular shape of the placenta was found in 30% of cases in lowlands and midlands and up to 43% of cases in the highlands, which was significantly higher than in low mountains primiparous (p<0,05).

Table 2 - Macroscopic characteristics of placentas in the study groups (absolute number and %)

Parameters	Primiparas			Multiparas		
	LM	MM	HM	LM	MM	HM
Rounded shape	9 (90%)	8 (80%)	7 (70%)	7 (70%)	7 (70%)	4 (57%)
Irregular shape	1 (10%)	2 (20%)	3 (30%)	3 (30%)	3 (30%)	3 (43%) *
Central attachment	9 (90%)	9 (90%)	9 (90%)	7 (70%)	6 (60%)	4 (57%) *
Paracentral attachment	1 (10%)	1 (10%)	1 (10%)	2 (20%)	3 (30%) *	2 (29%) *
Marginal attachment	0 (0%)	0 (0%)	0 (0%)	1 (10%)	1 (10%)	1 (14%)
Green colored membranes	1 (10%)	1 (10%)	3 (30%) *	2 (20%)	3 (30%) *	4 (57%) **, **

Note: * - difference compared to the low mountain group p<0.05

** - difference compared to the primiparous group p<0.05

Most primiparous women have a central attachment of the umbilical cord (90%) regardless of the altitude of residence). In multiparous women from the midlands and highlands there is a considerable frequency of the paracentral and marginal attachment ($p_{5-1} < 0,05$ and $p_{6-1} < 0,05$). In primiparous women from the lowlands, serving as a control group, the placenta is evenly blood-filled, fleshy, with clear boundaries, of whitish-pink color, with shiny colorless smooth membranes. Wharton's jelly is shiny, elastic. At the section placental tissue

looked succulent, of intense dark red color. We encountered one case of a single wedge-shaped ischemic infarct (see Table 3). Sclerosis and calcification was not observed. In primiparous women from midlands placenta was frequently unevenly blood-filled, there were a few cases of hemorrhagic and ischemic infarcts, intervillous thrombi. In 30% of cases hypertrophy of placenta was observed. Along with this, their lobules were smoothed, their boundaries were blurred.

Table 3 - Macroscopic developmental abnormalities of placentas in the study groups (absolute number and %)

Parameters	Primiparas			Multiparas		
	LM	MM	HM	LM	MM	HM
Hemorrhagic infarct	0 (0%)	1(10%)	2 (20%)	1 (10%)	2 (20%)	3 (42,7%)
Ischemic infarct	1 (10%)	1(10%)	1 (10%)	2 (20%)	2 (20%)	1 (10%)
Intervillous thrombi	0 (0%)	1(10%)	2 (20%)	3 (30%)	2 (20%)	4 (57,1%)
Hypotrophy of placenta	1 (10%)	1(10%)	3 (30%)	1 (10%)	2 (20%)	5 (71,2%)
Hypertrophy of placenta	1 (10%)	3 (30%)	2 (20%)	2 (20%)	3 (30%)	0 (0%)
Sclerosis of edematous villi	0 (0%)	0 (0%)	1 (10%)	3 (30%)	2 (20%)	4 (57,1%)
Calcification of placenta	0 (0%)	1 (10%)	1 (10%)	3 (30%)	2 (20%)	5 (71,2%)

In the highlands blood-filling of the placenta was clearly characterized as uneven, together with moderately blood-filled areas there were both sharply blood-filled and ischemic areas. In 20% of cases there was observed marked hypertrophy of the placenta, and in 30% it was hypotrophic, so that the average weight of the placenta was not significantly changed. In multiparous women from lowlands hemorrhagic infarcts, intervillous thrombi, sclerosis and calcification of the placenta were significantly more frequent than in primiparous women, with a rate of 30%. A similar morphological picture of the placenta was also observed in multiparous women from the midlands. The most pronounced macroscopic changes were

observed in placentas of women in high-mountain areas. The rate of hypertrophy of placentas was 71%. Placentas were characterized by a sharply uneven blood-filling, hemorrhagic and ischemic infarcts as counted together reached 52.7%, the rate of sclerosis was 57.1%, and the rate of calcification - 71.2% of observations.

The microscopic structure of the placenta had specific characteristics in primiparous and multiparous women, which grew more intense with increasing altitude of residence. Pathologic villous immaturity in primiparous was observed in only one case (the highlands), in multiparous this condition was seen with a frequency of 10% in the lowlands to 42.8% in the highlands, dissociated villous maturation was found both in primiparas from the highlands (20%) and in

multiparas from lowlands, midlands and highlands. Abnormal vascularization of the villi (5 capillaries per villus) was found with a greater frequency of 50% and higher in multiparas at any altitude and in primiparas at a rate of 30% in midlands and 60% in the highlands. With increasing

altitude of residence and parity, fibrin deposition and severity of mineral dystrophy (calcification) increased. Computerized morphometry showed the presence of structural distinctions of chorionic villi in primiparous and multiparous women (see Table 4).

Table 4 - Morphometric characteristics of chorionic villi

Parameters	Prim aras			Multiparas		
	LM	MM	HM	LM	MM	HM
Volume density of the villi	42.8±1.8	54.9±1.7 *	59.7±1.8 *	51.2±2.2 *	52.5±2.2	53.3±2.3 **
Length of villi (µm)	52.3±6.3	57.2±5.4	61.4±4.3	49.2±5.2	51.2±3.6	47.4±4.2

Volume density (the ratio of the area of villi to the area of chorionic space) in primiparas increased, according to altitude of residence, apparently due to the increased vascularization of the villi. The length of the villi had the same trend, but its increase was not significant ($p>0.05$). In multiparous women from lowlands the volume density of the villi was higher than in primiparous women, whereas in multiparous women from the highlands, on the contrary, it was lower, which we believe to be related to sclerosis and immaturity of the villi.

Table 5 - Levels of surface activity of amniotic fluid in the study groups

Parameters	Primiparas			Multiparas		
	LM (n=10)	MM (n=10)	HM (n=10)	LM (n=10)	MM (n=10)	HM a (n=7)
	1	2	3	4	5	6
ST _{min}	27,6 ±0,7	27,3 ±0,9	30,4±0,9*	27,5 ±1,1	32,4±0,9 *	33,1±1,2 *,**
ST _{max}	51.9±1.2	51.7±1.1	50.8±1.2	50.5 ±1.2	50.1±1.0	50.3±1.0
SI	0.62±0.4	0.62±0.4	0.50±0.3 *	0.59±0.4	0.49±0.3*	0.41±0.3 *,**

Note: * - difference compared to the low mountain group $p < 0.05$

** - difference compared to the primiparous group $p < 0.05$

Table 5 shows that surface activity (SA) levels did not differ in primiparous women from lowlands compared to the highlands. In primiparas from highlands SA was significantly reduced, as evidenced by a significant increase in ST_{min} and a decrease in SI ($p < 0.05$). In multiparous women from lowlands SA levels of amniotic fluid did not differ significantly from those of primiparous women. In the midlands and highlands the shifts in SA were more pronounced in multiparous than primiparous women.

Besides, a 33% decrease of the amniotic fluid SI in multiparas from high-mountain areas suggests immaturity of the lungs of newborns.

CONCLUSION

On the whole, the reduced surface activity of amniotic fluid and the macroscopic picture of placentas indicates subcompensated placental insufficiency multiparous living in high-mountain areas, which is a risk of neonatal respiratory distress syndrome.

Conflicts of Interest: None.

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