INTRODUCTION

Umbilical cord blood (UCB) is a source of the rare but precious primitive hematopoietic stem cells (HSC) and progenitor cells that can reconstitute the hematopoietic system in patients with malignant and nonmalignant disorders treated with myeloablative therapy ***(Bojanić and Cepulić , 2006)***.

The "naive" nature of UCB lymphocytes may explain the lower incidence and severity of graft versus host disease encountered in UCB transplant compared to the allogeneic transplant setting. Furthermore, UCB is rich in primitive CD16-CD56++ NK cells, which possess significant proliferative and cytotoxic capacities and can be expanded using IL-12 or IL-15, so as to mount a substantial graft versus leukemia effect ***(Cohena and Nagler, 2003)***.

UCB is an alternative source of hematopoietic stem cells for transplantation with success being associated with the total nucleated cell (TNC) count, CD34(+) cells and colony-forming unit-granulocyte-macrophage (CFU-GM) content infused ***(Wu et al., 2010)***.

TNC dose is an important influencing factor for hematopoietic stem cell engraftment in cord blood transplantation. An increased TNC dose may improve the success of cord blood transplantation ***(Liu et al., 2010)***.

Several obstetric factors influenced volume and TNC, such as greater infant and placental weight, predicting a better collection ***(Mancinelli at al., 2006)***.

UCB is usually discarded, and it exists in almost limitless supply. The blood remaining in the delivered placenta is safely and easily collected and stored ***(Bojanić and Cepulić, 2006)***.

Many UCB banks have been established worldwide to quickly provide high quality materials for transplantation. Moreover, a detailed set of standards for cord blood banking have been drawn to guarantee the quality of the products ***(Mancinelli at al., 2006)***.

AIM OF THE STUDY

This study is purposed to clarify the impact of maternal and neonatal factors on total nucleated cell count of collected umbilical cord blood of preterm delivery ≤ 36 weeks completed gestation.

UMBILICAL CORD

The umbilical cord is the cord that connects the developing fetus to the placenta. The umbilical cord develops from the yolk sac and allantois by the 5th week of fetal development and replaces the yolk sac as the nutrient supplier for fetus ***(Exalto, 1995)***. The umbilical cord averages 50-60 cm in length and about 2 cm in diameter at the end of gestation (***Di Naro et al., 2001)***.

The umbilical cord contains three blood vessels, one vein and two arteries which coil around the vein in a helical configuration **(Chaurasia and Agarwal, 1979)** (fig.1). The vein supplies the fetus with nutrient-rich oxygenated blood from the placenta and the arteries takes the nutrient depleted deoxygenated blood back to the placenta. The three blood vessels are insulated with a gelatinous substance called Wharton's jelly that protects these vessels and prevents their compression ***(Ferguson and Dodson, 2009)***.

Umbilical cord provides the means by which oxygen, carbon dioxide, steroids and other products are carried to and from the foetus, and it also allows free movement of the foetus within

the uterus and protects the umbilical blood vessels from mechanical injury ***(Abaidoo et al., 2008)***.

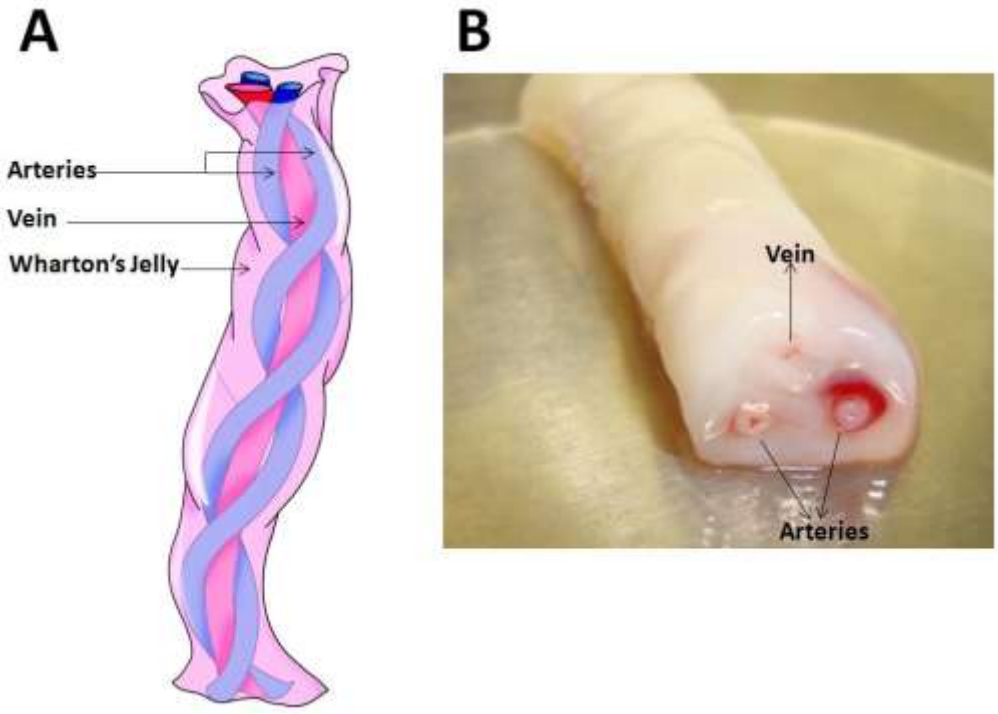

Figure 1. Anatomy of umbilical cord. (A) Umbilical cord has three blood vessels, two arteries and one vein that course through Wharton's jelly in a helical configuration. (B) Cross section of umbilical cord showing the vein and two arteries ***(Di Naro et al., 2001)***

❖ *Embryology of UC :*

The umbilical cord begins to form between four and six weeks of gestation, as the embryonic disc takes a cylindrical shape ***(Collins et al., 2002)***. At first, the embryo is a flattened disc interposed between the amnion at the dorsal surface and the yolk

sac at the ventral surface. By the end of the third week of development the embryo is attached to the placenta via a connecting stalk ***(Sepulveda, 2006)***.

Because the dorsal surface grows faster than the ventral surface, in association with the elongation of the neural tube, the embryo bulges into the amniotic sac and the dorsal part of the yolk sac is incorporated into the body of the embryo to form the gut ***(Van den Broek et al., 2005)***.

As the embryo enlarges, the unenclosed area of the ventral surface of the embryo becomes relatively smaller and even undergoes some constriction. This unenclosed region, at the junction of embryonic and extra-embryonic territories, is the primitive umbilicus ***(Sepulveda, 2006)***.

By the end of the 5th week the primitive umbilical ring contains 1) a connecting stalk within which passes the allantois (primitive excretory duct), two umbilical arteries and one vein; 2) the vitelline duct (yolk sac stalk); and 3) a canal which connects the intra- and extra embryonic coelomic cavities ***(Collins et al., 2002)*** (fig.2).

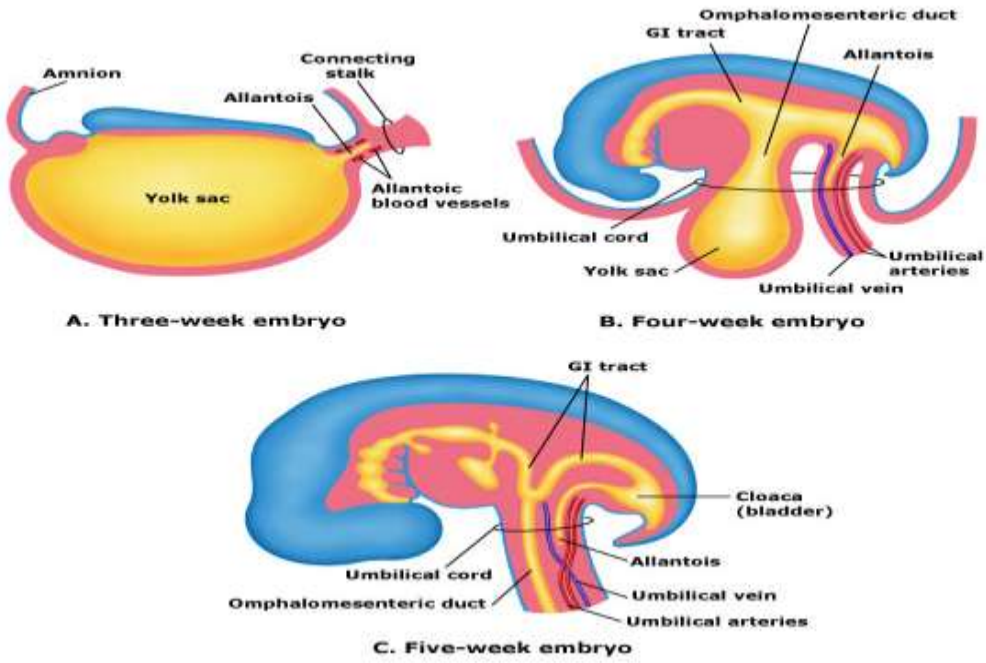

Figure (2): Embryology of umbilical cord (Collins et al., 2002)

By the 10th week the gastrointestinal tract has developed and protrudes through the umbilical ring to form a physiologically normal herniation into the umbilical cord ***(Van den Broek et al., 2005)***.

Normally these loops of bowel retract by the end of the third month. By about the middle of the third month, the expanding amnion obliterates the exo-coelom, fuses with the chorion laeve, and covers the bulging placental discs and the lateral surface of the body stalk, which is then called the umbilical cord, or funis. Thus, the umbilical cord comes into existence

through the expanding amnion applying itself about the body stalk and yolk stalk as it crowds them together ***(Collins et al., 2002)***.

❖ *Umbilical cord length and diameter :*

The length of the umbilical cord varies from no cord (achordia) to 300 cm, with diameters up to 3 cm. At term, the typical umbilical cord is 55 to 60 cm in length, with a diameter of 2.0 to 2.5 cm. About 5% of cords are shorter than 35 cm, and another 5% are longer than 80 cm ***(Valsamakis et al., 2006)***.

Though it is not fully understood what controls cord length, various authors correlate cord length with foetal activity and movement. It is suggested that sufficient space in the amniotic cavity for movement and the tensile force applied to the umbilical cord during foetal movements are two main factors that determine cord length ***(Benirschke, 2005)***.

An umbilical cord less than 40 cm is said to be short. Short umbilical cords are uncommon. They occur in approximately 6% of pregnancies. This shortness can be real or apparent (due to cord loops or entanglement). The pathogenesis of short umbilical cords remains unclear. One prominent hypothesis to explain the ontogeny of the umbilical cord is the "stretch hypothesis," which attributes the development of a short umbilical cord to intrauterine constraint ***(Van den Broek et al., 2005)***.

The presence of a short umbilical cord has been associated with ante-partum abnormalities and risk factors for complications of labour and delivery. ***Krakowiak et al. (2004)*** found out that infants with short umbilical cords were more likely to be female, have a congenital malformation, and be small for their gestational age. In this study, a short cord was associated with increased risk for maternal labour complications, including retained placenta and operative qvaginal delivery.

Long umbilical cords, defined as total length over 70 cm, are associated with a number of circumstances which can impact foetal life. Long cords have both maternal and foetal associations. Maternal factors include, systemic disease, delivery complications and increased maternal age. Foetal factors include cord entanglement, foetal anomalies, vertex presentation, increased birth weight, respiratory distress and male sex ***(Muppala et al., 2007).***

Infants with excessively long umbilical cords are found to be at a significantly increased risk of brain imaging abnormalities and/or abnormal neurological follow up ***(Muppala et al., 2007)***. In investigating the clinical significance of umbilical cord length in human pregnancies, ***Wu et al. (1996)*** found out that cord length was significantly related to birth weight. They however, found out that the umbilical cord length does not significantly correlate with

maternal age, gestational age, parity, foetal outcome or intrauterine foetal well-being.

❖ *Umbilical cord blood vessels :*

The umbilical cord normally contains of one umbilical vein and two umbilical arteries. Single umbilical artery (SUA), the most common anatomical abnormality of the umbilical cord, is found in 0.2-1.1% of singleton pregnancies and in 6-11% of multiple pregnancies. In this condition the umbilical cord is made up of two blood vessels, one vein and one artery, instead of the normal one vein and two arteries ***(van Dijk et al., 2002)*** (fig.3).

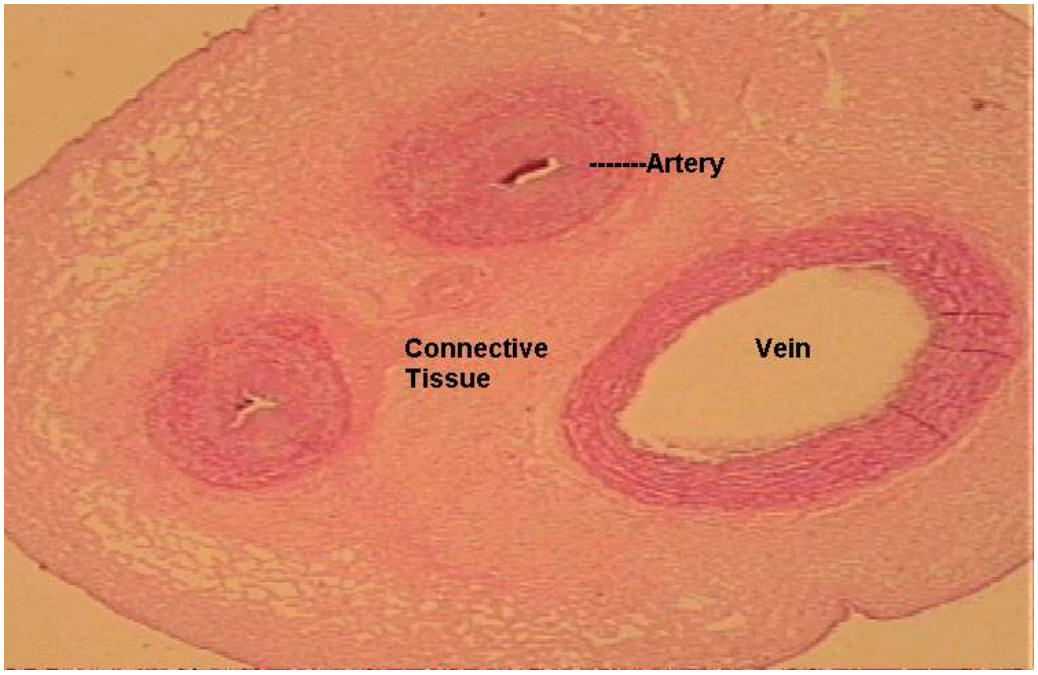

Figure (3): Umbilical cord vessels *(van Dijk et al., 2002)*

It is believed to be caused by atrophy of previously normal artery or agenesis of one of the umbilical arteries ***(Valsamakis et al., 2006)***. The selection process of the missing (or existing) vessel is likely to be random, even though a right single artery is slightly more common. Of infants with single umbilical artery 20% or more are reported to have associated foetal anomalies including cardiovascular abnormalities, gastrointestinal tract defect, esophageal atresia, a variety of renal defects and multiple anomaly syndromes ***(Martinez-Frias et al., 2006)***.

5-20% perinatal mortality rate has been reported in association with single umbilical artery and includes foetuses with severe congenital anomalies and chromosomal defects ***(Valsamakis et al., 2006)***.

❖ *Umbilical cord coiling :*

The three blood vessels of the umbilical cord pass along the length of the cord in a coiled fashion. Several theories have been proposed to explain the umbilical cord twist including those that interpret the twist as inherent to the cord itself, and those that explain the twist as a result of active or passive rotation of the foetus. Regardless of its origin, umbilical coiling appears to confer turgor to the umbilical unit, producing a cord that is strong but flexible ***(Gupta et al., 2006)***.

Umbilical cord coiling index (UCI) has been reported to be approximately 1 coil/5 cm of umbilical cord length or 0.20 (SD 0.07) ***(Machin et al., 2000)***. However, ***De Laat et al. (2005)*** showed it to be 0.17 (SD 0.009) while ***Gupta et al. (2006)*** in his study found it to be 0.31 (SD 0.08) complete spirals per centimeter. The total number of coils for any particular cord is believed to be established early in the gestation ***(van Dijk et al., 2002)***.

The pattern of coiling develops during the second and third trimesters, presumably due to snarls in the cord, and this coiling change as pregnancy advances. The presence of a mixed coiling pattern and even reversal of the coiling direction in third trimester have been shown ***(Quin et al., 2002).***

The helical disposition of the umbilical cord affords it an elastic property which enables it to resist external forces such as torsion, compression and tension that might compromise the umbilical vascular flow. The coiled umbilical cord acts like a semi erectile organ that is more resistant to snarling torsion, stretching and compression than non-coiled one. Hyper coiled cords often have more thrombi in placental surface veins because the flow is more sluggish, and when coiling becomes excessive, the foetus can strangle circulation in the cord vessels ***(Benirschke, 2005)***.

Meta-analysis by ***De Laat et al. (2005)*** pointed out the fact that hypo coiling is associated with increased incidence of foetal demise, intrapartum foetal heart rate deceleration, operative delivery, foetal distress and chorio-amnionitis. ***Kashanian et al., (2006)*** also showed that neonatal weight in normocoiled and hypocoiled cords were higher than that of hyper coiled cords.

❖ *Umbilical cord insertion :*

The location of umbilical cord attachment to the foetus and placenta is also important. The umbilical cord insertion into the placenta is described as central, eccentric, marginal, or velamentous as it relates to the chorionic plate ***(Valsamakis et al., 2006)***.

Typically, the umbilical cord inserts at the center or near the center, also known as central or eccentric insertion respectively. About 90% of cord insertions are central or eccentric and about 7 percent of umbilical insertions occur at the placental margin ***(Yetter, 1998)***.

Though the factors that determine the site of insertion of the umbilical cord on the placenta are not entirely known, some researchers associate it to errors in implantation of the blastocyst into endometrium ***(Baergen et al., 2001)***.

According to ***Collins et al. (2002)***, these abnormal cord insertions occur as a result of the 'migration' and 'dissolution' of the placenta from its original site, a process called trophotropism, which sometimes occurs during its development. In this case, there appears to be a relocation of the placenta, which dissolves, leaving the amnion remaining, which can then be the insertion site of the umbilical cord. This results in the umbilical cord and placental end being connected at the edge of the placenta (marginal insertion) and a membranous insertion (velamentous insertion) ***(Baergen et al., 2001)***.

Marginal insertions, according to ***Yetter (1998)***, are generally benign. Marginal cord insertions are more common than velamentous cord insertion. It occurs in approximately 5 % of pregnancy ***(Benirschke, 2005)***. Marginal cord insertion has also been associated with foetal growth impairment and preterm delivery ***(Valsamakis et al., 2006)***.

A velamentous insertion is reported to occur in approximately 1–2% of singleton Pregnancies ***(Valsamakis et al., 2006)***. However, the prevalence of this finding is higher in multiple gestations ranging from 13% to 21% for twins ***(Machin, 1997)***. It is more frequently identified in mono-chorionic twin gestations and has been associated with the twin-to-twin transfusion syndrome. Velamentous insertion has been diagnosed

by ultrasonography with a sensitivity of 67% and specificity of 100% in the second trimester; first trimester diagnosis is also possible ***(Sepulveda, 2006)***.

Potential complications associated with velamentous insertion include miscarriage, prematurity, low birth weight, foetal malformation, perinatal death, low Apgar scores, and retained placenta ***(Sepulveda et al., 2003)***. However, the most relevant is vasa previa, a condition in which the velamentous vessels run in the lower uterine segment unprotected by the Wharton jelly ***(Sepulveda et al., 2003)***. These vessels are prone to compression and bleeding preferentially at the time of delivery and may cause unexpected foetal death due to hypoxia or exsanguinations ***(Oyelese et al., 2004)***.

❖ *Umbilical cord blood cells :*

Human umbilical cord blood (HUCB) cells are an attractive source for cell transplantation because these are easy to harvest and survive long-term cryopreservation. However, whole UCB samples contain stem and progenitor cells of mixed lineage (blood and mesenchymal) as well as mature blood cells, resulting in cell cultures with multiple cell types. Several groups have reported the presence of mesenchymal progenitor cells from UCB.