

**Histomorphometry of umbilical cord and its vessels in natural and assisted reproduction**V. Ananthi<sup>1</sup>, D. Rajkumar<sup>2</sup>, V. Muniappan<sup>3</sup>Tutor<sup>1</sup>, Dean<sup>2</sup>, Faculty of Medicine, Professor and Head<sup>3</sup>, Department of Anatomy, Rajah Muthiah Medical College, Annamalai University, Chennai, Tamilnadu*(Received: August 2019      Revised: October 2019      Accepted: December 2019)*Corresponding author: **D. Rajkumar**. Email: kumaran.cm@gmail.com**ABSTRACT**

**Introduction and Aim:** Assisted reproductive technique (ART) is an invaluable gift for infertile couple who failed in various infertility treatments. However, there are many aftereffects after assisted reproduction like preterm birth, small for gestational age, congenital anomalies etc. Umbilical cord is of fetal origin, which connects the fetus to placenta. Therefore, changes in the histomorphometry of umbilical cord and its vessels may affect the fetus and its growth. Our present study aimed to analyze the histo-morphometric parameters of umbilical cord and its vessels in natural conception and in assisted reproduction.

**Materials and Methods:** 30 placentas (with umbilical cord) of natural conception and 30 placentas (with umbilical cord) of assisted reproduction (ICSI) were collected immediately after delivery in and around Chidambaram. Umbilical cord bits were fixed in buffered formalin and underwent routine tissue processing procedure. 5 to 10 microns of tissue sections were taken and stained in H & E stain, Verhoeff stain and MTS stain. Histomorphometric parameters of umbilical cord and its vessels were measured in Olympus microscope using ocular micrometer.

**Results:** There was a reduction in the areas of umbilical cord and its vessels in assisted reproduction when compared to natural conception.

**Conclusion:** These results would be useful for neonatologists who handle the newborns of ART mothers.

**Keywords:** Umbilical cord; umbilical artery; umbilical vein; histomorphometry; connective tissue.

**INTRODUCTION**

Assisted reproduction is a technology where both egg and sperm are handled. It involves surgically removing eggs from women's ovaries, combining them with sperm in the laboratory and returning them to the women's body or donating them to another woman.

**Intracytoplasmic sperm injection**

Spermatozoa sometimes fail to fertilize even when they were artificially placed in close proximity to eggs during conventional *in vitro* fertilization (IVF). Fertilization failure in IVF is particularly common where there are gross abnormal semen parameters or number of spermatozoa is insufficient. The placing of spermatozoa beneath the zona has yielded consistent result. The intracytoplasmic sperm injection procedure entails the deposition of single spermatozoon directly into the cytoplasm of the oocyte, thus bypassing the zona pellucida and oolemma. Retrieval of low number of oocytes represents a further indication for this procedure (1).

Assisted reproductive technique (ART) is a great gift for infertile couple those who are exhausted by taking various infertility treatments and failed in it. But there are many consequences in fetal growth like preterm birth, small for gestational age etc. There are very few studies about placenta and the connecting cable umbilical cord changes in assisted reproduction.

Umbilical cord is the only connecting link between embryo and the placenta. The embryo remains attached to the trophoblast only by extra-embryonic mesoderm into which the coelom does not extend. This extra-embryonic mesoderm forms the connecting stalk. This connecting stalk is only formed as umbilical cord (2).

As embryo grows, the attachment of connecting stalk moves towards the ventral aspect of the embryo. It is attached in the region of umbilical opening. The tube of amnion, two arteries, one vein and Wharton's jelly (mesoderm) constitutes umbilical cord. Initially there are two arteries and two veins, but later right vein disappears. Umbilical cord is when fully developed, on an average some 50cm long and 1-2 cm in diameter are attained. But the length is subjected to great variation (20-120 cm). The cord usually attaches to centre of the placenta. In few cases velamentous insertion is observed (i.e. into the membranes) and may be vulnerable to injury (3). Umbilical cord plays an important role in maintaining and regulating fetoplacental circulation and thus in fetal nutrition and wellbeing (4). Umbilical vessels within the umbilical cord are not supplied by vaso-vasorum and thus depend on their oxygen supply making them more vulnerable to changes in hemodynamic condition. Umbilical cord is full of fetal origin and changes in the umbilical cord morphometry may affect the fetus. Our present study aimed to analyze the histomorphometry of

umbilical cord, its vessels and connection tissue in natural conception and in assisted reproduction.

**MATERIALS AND METHODS**

Thirty placentas (with umbilical cord) of natural conception and 30 placentas (with umbilical cord) of assisted reproduction were collected from Rajah Muthiah Medical College, OBG Department and from private fertility center in and around Chidambaram. In ART 12 cases were dichorionic, diamniotic twins. So totally the umbilical cords of ART were 42. Human ethical committee clearance was obtained for sample collection. Patient history was collected using proper proforma. Umbilical cord bits were taken five centimeters away from placental end. Cord bits were fixed in buffered formalin. Cord bits well processed and two blocks were made for each specimen. 5 to 10 microns thick sections were made and stained by hematoxylin and eosin stain, Verhoff stain and Masson trichrome stain. Various histomorphometric parameters of umbilical cord were measured by ocular micrometer in Olympus microscope.

**The following parameters were measured:**

**1. Umbilical cord**

- a. Total umbilical cord area

- b. Connective tissue area

**2. Umbilical artery (A)**

- a. Total area of umbilical artery
- b. Lumen area of umbilical artery
- c. Outer layer area of umbilical artery
- d. Inner layer of umbilical artery
- e. Wall area of umbilical artery

**3. Umbilical artery (B)**

- a. Total area of umbilical artery
- b. Lumen area of umbilical artery
- c. Outer layer area of umbilical artery
- d. Inner layer of umbilical artery
- e. Wall area of umbilical artery

**4. Umbilical vein**

- a. Total area of umbilical vein
- b. Lumen area of umbilical vein
- c. Wall area of umbilical vein

**Statistical analysis**

Statistical analysis was performed using SPSS. The data obtained from the study were compiled and expressed as mean ± standard deviation. Mann-Whitney test was performed. Two tailed ‘t’ test were done. Z value and P values were also tabulated. P value of < 0.05 was taken as significant.

**RESULTS**

**Table 1:** Clinical characteristics of normal and assisted reproductive pregnancies

S. No.	Parameters	Normal (mean)	ICSI (mean)
1	Age (years)	28.33	32.93
2	Hb (g%)	11.07	11.77
3	Gestational age (weeks)	37.033	36.16
4	Weight of baby (Kg)	2.92	2.417
5	Mode of delivery VD/caesarean	30/2	0/30
6	Abgar score	9/10	8/10

Mean age of mother increased in assisted reproduction when compared to natural conception. But mean gestational age and weight of baby were reduced in assisted reproduction (Table 1).

**Table 2:** Histomorphometry of umbilical cord

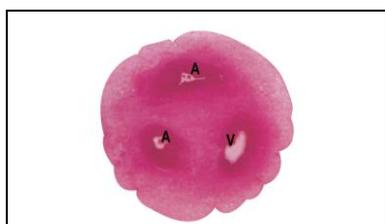
S. No.	Parameters	Normal mm <sup>2</sup>			ICSI mm <sup>2</sup>			Z value	P value
		No. of cases	Mean	Std	No. of cases	Mean	Std		
1	Total Umbilical cord area	30	57.16	0.95	42	49.88	21.7	1.84	0.065
2	Connective Tissue area	30	45.63	15.97	42	40.08	20.52	1.67	0.09

P value <0.05 is significant

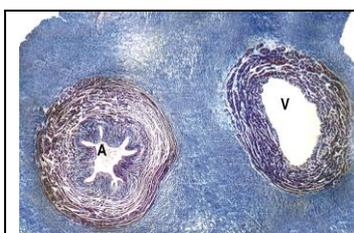
Our present study showed that the total umbilical cord area was 57.17 mm<sup>2</sup> in natural conception. It was about 49.88 mm<sup>2</sup> in assisted reproduction (table 1). Connective tissue area of umbilical cord was 45.63mm<sup>2</sup> in natural conception. It was about 40.08 mm<sup>2</sup> in ART (Table 2).

Histological examination of umbilical cord showed that there was a well-defined single layered squamous amniotic epithelium. Deep to that was a mucoid connective tissue i.e., Wharton’s jelly within

the jelly are umbilical vessels (Fig. 1). Two arteries and a single umbilical vein were present in umbilical cord. The arteries possess no elastic lamina and doubly layered smooth muscle wall vein has inner elastic lamina (Fig. 2 and 3). The umbilical vein has a larger diameter as compared to arteries and has a thin single layer of muscle coat (Fig.2 and 4). The umbilical artery possesses thick double-layered smooth muscle coat when compared to umbilical vein (Fig. 5).



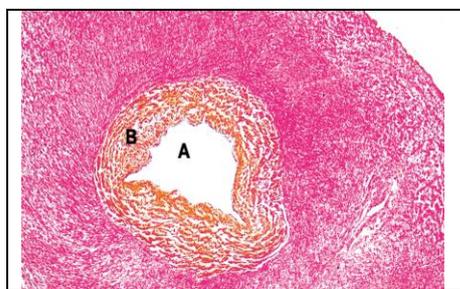
**Fig. 1:** Full view of Umbilical cord with two arteries(A), one vein(V). H&E stain



**Fig. 2:** Umbilical artery (A) and Vein (V) of Umbilical cord. Masson trichrome stain,10x



**Fig. 3:** Umbilical artery. (A) Lumen area, (B) Inner layer area, (C) Outer layer area. H & E stain,10X



**Fig. 4:** Umbilical vein. (A) Lumen area, (B) Wall area. Verhoeff stain, 10X



**Fig. 5:** Umbilical artery. (A) Inner layer area, (B) Outer layer area H & E stain, 40X

**Table 3:** Histomorphometry of umbilical artery (A)

S. No.	Parameters	Normal (mm <sup>2</sup> )			ICSI (mm <sup>2</sup> )			Z value	P value
		No. of cases	Mean	Std	No. of cases	Mean	Std		
1	Total artery area	30	3.12	1.66	42	2.92	1.96	1.17	0.239
2	Lumen Area	30	0.28	0.37	42	0.23	0.23	0.08	0.936
3	Outer layer area	30	1.94	0.7	42	1.63	0.77	2.09	0.37
4	Inner layer area	30	0.59	0.42	42	0.66	0.36	1.65	0.098
5	Wall area (inner+outer)	30	2.84	1.51	42	2.69	1.8	1.19	0.67

P value <0.05 is significant

**Table 4:** Histomorphometry of umbilical artery (B)

S. No.	Parameters	Normal (mm <sup>2</sup> )			ICSI (mm <sup>2</sup> )			Z value	P value
		No. of cases	Mean	Std	No. of cases	Mean	Std		
1	Total artery area	30	4.05	2.25	42	3.52	3.18	1.82	0.067
2	Lumen Area	30	0.411	0.6	42	0.35	0.45	0.131	0.89
3	Outer layer area	30	2.28	1.48	42	1.68	0.89	1.86	0.063
4	Inner layer area	30	0.67	0.56	42	0.77	0.45	1.78	0.07
5	Wall area (inner+outer)	30	3.64	1.86	42	3.17	2.84	2.16	0.03

P value <0.05 is significant

**Table 5:** Histomorphometry of umbilical vein

S. No.	Parameters	Normal (mm <sup>2</sup> )			ICSI (mm <sup>2</sup> )			Z value	P value
		No. of cases	Mean	Std	No. of cases	Mean	Std		
1	Total umbilical Vein area	30	4.36	2.11	42	3.33	1.93	3.06	0.002
2	Lumen Area	30	1.41	1.4	42	0.95	0.98	1.73	0.08
3	Wall area (inner+outer)	30	2.94	0.95	42	2.37	1.35	2.99	<b>0.003</b>

P value <0.05 is significant

**DISCUSSION**

Assisted reproductive technique is a great boon to infertile couple who are exhausted by using various infertility treatments and failed in it. There are some

aftereffects in assisted reproductive techniques because of artificial manipulation of gametes. The state of vascular system of mother and of placenta has exerted great influence on intrauterine growth of fetus. The umbilical cord is a connecting cable

between fetus and placenta. It acts as a carrier and mediator between fetus and placenta. The arteries within it return poorly oxygenated blood to the placenta while the vein carries oxygenated blood from this tissue to the fetus (5-7).

The Wharton's jelly is a metabolically active tissue involved in fluid exchange between amniotic fluid and umbilical vessels. Torsion and fibrosis of Wharton's jelly and thickening of vascular wall, which obstructs the fetoplacental circulation leading to anoxia and fetal death (8, 9), usually accompany the lean umbilical cords. Reduced Wharton's jelly could be due to fetal starvation, dehydration and poor maternal nutrition. Absence of Wharton's jelly may result in antenatal fetal death (10). These reduced Wharton's jelly may cause compression of umbilical vessels (11). According to Barnwal *et al.*, the total umbilical cord area was  $51.56 \pm 2.34 \text{ mm}^2$  and connective tissue area was  $42.27 \pm 2.12 \text{ mm}^2$  (12). In our present study umbilical cord area were  $57.17 \pm 16.77 \text{ mm}^2$  in natural conception and  $49.88 \pm 21.7 \text{ mm}^2$  in assisted reproduction. Connective tissue area of umbilical cord was  $45.62 \pm 15.97 \text{ mm}^2$  in natural conception  $40.08 \pm 20.52 \text{ mm}^2$  in assisted reproduction. There was significant reduction in total umbilical cord area and connective tissue area of umbilical cord in assisted reproduction (Table 2). In Kotrannavar *et al.*, study, the total vein area was  $2.16 \pm 0.54 \text{ mm}^2$  in control group and  $1.55 \pm 0.45 \text{ mm}^2$  in IUGR cases (13). Our present study showed  $4.36 \pm 2.11 \text{ mm}^2$  in natural conception and  $3.33 \pm 1.93 \text{ mm}^2$  in assisted reproduction (Table 5). There was a significant reduction of vein area in assisted reproduction.

In Shaima *et al.*, study, the total area of umbilical vein was  $2.09 \pm 1.43 \text{ mm}^2$  in control group and  $3.1 \pm 3.32 \text{ mm}^2$  in pre-eclamptic group, whereas lumen area of vein was  $0.24 \pm 0.04 \text{ mm}^2$  in control group and  $5.05 \pm 0.40 \text{ mm}^2$  in pre eclamptic group (14). In our present study umbilical vein area was  $4.36 \pm 2.11 \text{ mm}^2$  in natural conception and  $3.33 \pm 1.43 \text{ mm}^2$  in assisted reproduction. Lumen area of umbilical vein was  $1.416 \pm 1.4 \text{ mm}^2$  in natural conception and  $0.95 \pm 0.98 \text{ mm}^2$  in assisted reproduction. Wall area of umbilical vein was  $2.94 \pm 0.95 \text{ mm}^2$  in natural conception and  $2.37 \pm 1.35 \text{ mm}^2$  in assisted reproduction (Table 5). All the parameters of umbilical vein were reduced in assisted reproduction. There was a significant reduction in umbilical vein wall area and total umbilical vein area. According to Blanco *et al.*, study, the outer layer area and Inner layer area of umbilical artery was increased in pathological conditions like IUGR, preeclampsia, gestational diabetes mellitus and antiphospholipid syndrome when compared to normal group of placentas (15). Inner layer area of umbilical artery (A) and umbilical artery (B) increased in assisted reproduction when compared to natural conception

(Table 3 and 4). According to Inan *et al.*, study, there was a significant reduction in the total umbilical vein area, wall thickness of vein and narrowing of vein lumen was observed in preeclampsia cases. The thickness of arteries was 20% increase in preeclampsia group (16). In Junek *et al.*, study, the thickness of umbilical arteries (both intima and media) was 15% increase in preeclampsia. The thickening of tunica intima was due to the emigration of smooth muscle cells towards endothelium and splitting of internal elastic lamina. This emigration was due to rise in the sulphated glycosaminoglycan in umbilical arteries (17). In Bruch *et al.*, study, they found that there was a reduction in the Wharton's jelly area, luminal and wall areas of umbilical vein in Intra uterine growth restriction (IUGR) cases. Hypoblastic umbilical vessels are associated with an increase in the placental vascular resistance which in turn caused by chronic defect in the placental blood flow (18). There was a strong association between lean umbilical cord and the small for gestational age (SGA) infant (11).

We provide the histo-morphometric parameters of umbilical cord and its vessels in natural conception and assisted reproduction. Overall, there was a reduction in the areas of umbilical artery (A), umbilical artery (B), umbilical vein, lumen areas of all the vessels and areas of total umbilical cord and connective tissue. However, statistically significant changes were seen in outer layer area of artery (A), wall area of artery (B), umbilical vein area and umbilical vein wall area. There was an increase in the inner layer of umbilical artery (A) and (B) in assisted reproduction when compared to natural conception.

The changes in wall and luminal areas may be partially explained by the fact that throughout the last 2 weeks of pregnancy, the cord vessels show increasing responsiveness to mechanical irritation (19). Umbilical vessels are very sensitive to various endocrine mediators, such as serotonin, angiotensin, prostaglandins and oxytocin (20-22).

Our present study showed that there was significant reduction in areas of umbilical cord, connective tissue and its vessels in assisted reproduction. These changes may reflect over fetus and its growth. It may cause preterm birth, small for gestational age and congenital anomalies of fetus. To the best of our knowledge, this is the first study in the umbilical cord of assisted reproduction. These histo-morphometric details would be useful for neonatologists and pediatricians who handle the newborns of ART cases.

**CONFLICTS OF INTEREST:** None

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