

Treatment of Cervical Insufficiency Abortion By Autologous Human Peripheral Blood: Mononuclear Cell, Modern Trend

¹Ali Farid Mohamed Ali and ²Laila Ali

¹Heliopolis Research Center, Cairo Egypt.

²Department of obstetrics and Gynaecology Ain Shams University Cairo Egypt.

elshayb1950@yahoo.com

Abstract: To test the Efficacy of outpatient intracervical injection of peripheral blood mononuclear cells in the treatment of habitual abortion cervical insufficiency. Pilot study Heliopolis research center and Heliopolis Hospital, Cairo, Egypt Preparation of peripheral blood mononuclear cells, transvaginal ultrasound, IL-8, collagenases in the cervical mucus, Aquaporins in the cervical smears before PBMC injection at the time of the delivery. Primary outcome was delivery of full term fetus. Obstetric outcome Full term delivery occurred in 48 cases (96%), abortion 1 case (2%), Preterm delivery 1 case (2%), vaginal delivery in 40 cases (80%), cesarean section in 9 cases (18%). No fetal or maternal complication, were reported. Treatment of cervical insufficiency habitual abortion by cervical injection of autologous human peripheral blood mononuclear cell (PBMCS) is safe, effective, and cheap with positive fetal effect and no fetomaternal complications, but more cases and randomization is needed before elucidation the effectiveness of the procedure.

[Ali Farid Mohamed Ali and Laila Ali **Treatment Of Cervical Insufficiency Abortion By Autologous Human Peripheral Blood: Mononuclear Cell, Modern Trend.** *Life Sci J* 2013;10(4):2424-2428]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 324

Keywords: Cervical insufficiency, Abortion, PBMC, Aquaporin, IL8, Collagenase, Cerclage.

1.Introduction

The first recognition of cervical incompetence was reported in the literature in 1658(1). The authors noted that: The second fault in women which hindered conception is when the seed is not retained or the orifice of the womb is so slack that it cannot rightly contract itself to keep in the seed. The fibers of the womb are broken in pieces, one from another and the inner orifice of the womb overmuch slackened". Despite the description of this condition, a surgical approach for treatment did not emerge until nearly 300 years later.

Treatment of cervical insufficiency abortion is by cerclage which either done vaginally (Schirodkar, McDonald) or abdominally and laparoscopically. Due to many complications arise from Cerclage a need for non-invasive or minimally invasive procedure is urgent. Cerclage is not an innocent procedure it is associated with an increased risk of premature preterm rupture of membranes, bleeding, and intrauterine infection, cerclage may also cause severe pain and inconvenience throughout pregnancy (2)As mononuclear. cell is a potential regulator of cell proliferation (3,4)Stimulate progesterone by luteal cells(5), promote embryo invasion(6), stimulate Adenosinetriphosphate(7), improving mitochondrial respiration(8),express HCG receptors, strong anti-microbial(9) Hence comes its application in cervical injection.

2. Material and Methods

Fifty pregnant women at a period of gestation between 8-10 weeks to exclude fetal causes of abortion. Informed consent was taken. The mean age of women was 30 ± 3.5 years, a mean number of abortion was 8.2 ± 2.6 were enrolled in the study, inclusion criteria were historical and radiological features of cervical insufficiency. Traditionally, historical features were used to make the diagnosis of cervical insufficiency (10) which were (i) history of two or more second trimester pregnancy losses (excluding those resulting from preterm labor or abruption); (ii) History of losing each pregnancy at an earlier gestational age; (iii) History of painless cervical dilatation of up to 4-6 cm; (iv) absence of clinical findings consistent with placental abruption; (History of cervical trauma caused by cone biopsy); (vi) intrapartum cervical lacerations; or (vii) excessive forced cervical dilation during pregnancy termination. Transvaginal ultrasound for measurement of absolute cervical length, cervical effacement after the cervical injection was done every two weeks.

Exclusion criteria:

Previous cerclage, other causes of abortion medical and genetic disorder, previous progestational drugs, viral hepatitis and blood diseases. Ultrasound done before the procedure for diagnosis and then every 2 weeks after the

procedure, also cervical smear for aquaporin and cervical mucous for detection of IL8, collagenase were done before the procedure and at the time of delivery. Fetal evaluation by Apgar score and fetal weight was done.

Detection of IL8 in cervical mucus before the procedure and at the time of delivery (11), Collagenase(12,13,14), Detection of aquaporins type AQP3, AQP4, AQP5 in the cervical smear cell by immunohistochemistry (15,16)

For detection of Aquaporins:

Evaluation of staining intensity was performed by using a grading scale from 0 to 3 where 0 = no staining, 1 = faint staining, 2= moderate staining and 3 = intense staining. The number of stained cells were similar in all smears, two observers each unaware of the identity of slides evaluated the staining intensity the average value from the two observers was calculated (17)

Preparation of Autologous Peripheral Blood Mononuclear Cells:

Blood samples were obtained from individual patients and PBMCs (1×10^7 cells) were isolated by Ficoll-hypaque Centrifugation as described previously(5) After isolating PBMCs, the cell suspension was gently administered as an outpatient using sedation in the form of 15 mg pethidine and 10 mg pentazocain intravenously, The administration was in the cervical wall at any

site of the cervix no difference whether anterior or posterior not passing through the cervical canal.

The amount of cell suspension differ from patient to patient. The senior author introduce a new equation which is the amount of cell suspension to be injected= length of cervix x inner-inner diameter of the cervix.

Ethics:

Informed Consent and explaining all the details of the procedure to the patients, and all procedures involving human subjects complied with the declaration of Helsinki 1975 and revised in 2000, again all the procedures accepted from the ethical committee of Heliopolis research center and Heliopolis Hospital.

Data analysis:

Difference between IL8, collagenase, AQP3, AQP4, AQP5 before activated PBMC and at the time of delivery were analyzed by two tailed t-test, chi-square test was used for comparison a difference of <0.05 was considered significant.

3. Results

Fifty pregnant women at a period of gestation between 8-10 weeks were subjected to autologous human peripheral blood mononuclear cells injected in the cervical wall the results were summarized in the following tables.

Table (1): Cervical mucus collagenase, IL-8 before autologous human peripheral blood mononuclear cell " (PBMC)" cervical injection and at time of delivery (n= 50).

Character	Before PBMC Injection	After delivery	P value
IL-8	6.11±2.1	2.2±0.5	$P<0.05$
Collagenase	5.21±1.5	11.9±1.8	<0.05

Table (2): Staining intensity of AQP3, AQP4, AQP5 in cervical smear. Before PBMC and after Delivery (no. 50):

(Type AQP)	Before PBMC Injection	After Delivery	P Value
AQP3	4.1±2.99	2.1±1.88	<0.5
AQP4	5.2±3.18	3.1±2.99	<0.05
AQP5	6.5±4.1	2.1±1.56	<0.001

Table (3): Obstetrics Outcome after PBMC cervical Wall injection (no. 50).

Character	No.	%
Full Term	48	96%
Abortion	1	2%
Preterm	1	2%
Vaginal delivery	40	80%
Casarian section	9	18%
Evacuation of abortion	1	2%

Table (4): Fetal assessment (n = 49)

character	Mean ± SD
Birth Weight	3.995±0.805
APGAR score First minute	8±0.1
APGAR score Fifth minute	9±0.2

No prenatal loss, or any other fetal congenital malformation were recorded

4. Discussion

Treatment of cervical insufficiency abortion is by cerclage which done either vaginally (Shrodkar or McDonald) or abdominally and laparoscopically.

Women with cerclage had more hospitalization during pregnancy, lower birth weight and had a higher of maternal peripheral fever (18).

Some investigators (19) Also failed to show any benefit from cerclage in a large cohort of 506 women according to the largest randomized study was performed by the medical research council/ royal collage of obstetricians and gynecologists MRC/RCOG, women who were assigned to cerclage received more tocolytic medications (34% vs 27%) than in women without cerclage, and had a higher rate of antepartum hospital admissions (37% vs 29%). Puerperal fever was also more commonly after cerclage (6% vs 3% $p=0.03$).

In A meta-analysis (20). pooled the results of four trials that together summarized 2062 women. There was no difference in the total pregnancy and early < 24 weeks pregnancy loss rates (relative risk RR 0.86, 95%). There was no beneficial effect of cerclage (RR 1.29, 95%, CI 0.67-2.49), there was also no difference in perinatal death (RR 0.8, 95%, CI 0.48-1.36) or mean gestational age between cerclage and non cerclage group. So, in summary it appears that the use of cerclage (21,22) in women with various risk factor of spontaneous preterm birth has little clinical benefit, but is associated with more medical complication and interventions (28)

Due to the above situation regarding cerclage vaginally or abdominally (28). We introduced for the first time in the literature a new technique based on cervical injection of autologous human peripheral blood mononuclear cell. we reached to a full term pregnancy rate 96%, 2% abortion, 2% preterm labour (table 3). In our work we found cesarean section rate was 18% and vaginal delivery rate was 80% (table 3). Mean birth weight 3.995 ± 0.805 (table 4) and the mean Apgar at one minute and five minutes was 8 ± 0.1 , 9 ± 0.2 . We reported here no case of prenatal loss. To explain the results of our work a serial ultrasound were done postoperatively we found a normalize of the cervical internal assessment at the time of delivery than before PBMC injection.

The biochemical basis of our results is based upon statistically diminished collagenase enzyme (Table 1) at the time of delivery meaning that the technique act by stimulating a new collagen fiber and increase the number of collagen number

fibers in the cervix it was demonstrated (25) that increase in collagen type I which is a rigid fibril collagen and diminished collagen type III which is elastic and thin and result in more compliant tissue. The ratio between collagen I and III determines the characteristics of the tissue, Again *it was* demonstrated (25) that increase in the ratio between collagen type I and III in cervical biopsy taken at time of delivery, increased the gap junction proteins and up regulation of useful genes and positive effect on amniotic fluid meaning that it increase the amniotic fluid in cases of oligohydramnios (25)

Regarding cervical mucus IL-8 we demonstrated a statistically significant decrease at the time of delivery. IL-8 is a marker of infection meaning that our technique reduce the incidence of infection contrasting to cerclage operation in which infection is raised, the reduction of infection is based upon the fact that (PBMC) is a major source of proteinase nexin-1 which is a strong antimicrobial (9).

We studied aquaporin in the cervical smear for the first time in the literature before the procedure and at the time of delivery we found the statistically significant decrease in AQP3 and AQP4 and statistically highly significant decrease in aquaporin meaning that our technique works by other mechanism which through the reduction of expression of AQP. This means that the cervix become more rigid and this attributed partially to success of this new procedure. Again we should stress the point that there were differential expression of AQP it is less in the side of AQP5 which means that it is the major AQP responsible for the change in the elasticity of the cervix.

It was demonstrated that PBMC improved skin graft survival (27). And PBMC is a rich source of early pregnancy factor which has an immunosuppressive and growth factor properties (28) Regarding the method of delivery we demonstrated in (Table 3) vaginal delivery 80%, Cesarean section 18%, the low incidence of cesarean sections gives another advantage for this new procedure in comparison to the high incidence of cesarean section in cerclage group (28) this means that cervical dystocia which was encountered in cerclage is not encountered in our procedure. The cost of this procedure is only 5 dollars which in comparison to cerclage is more advantageous these points added to the cost benefit ratio of this procedure. So the advantage of our technique in comparable to cerclage were: Outpatient, nonsurgical, no need for antibiotics, no anesthesia, positive effect on fetus, strong antimicrobial (No tape and infection related to the tape as in cerclage), cheap (5 dollars), easy learning curve, promote

fibrosis(25), up regulation of useful genes(25), stimulation of gap junction protein(25)), stimulate type I collagen(25), increase ratio collagen I / collagen III, reduce type III collagen, positive electron microscope effect(25).

Conclusion

Treatment of cervical insufficiency by cervical injection of autologous human peripheral blood mononuclear cell (PBMCS) is safe, effective, and cheap with positive fetal effect and no fetomaternal complications, but more cases and randomization are needed before elucidation the effectiveness of the procedure.

Corresponding Author

Ali Farid Mohamed Ali, MD

Professor, Exchairman of Obstetrics and gynecology faculty of medicine Ain Shams univeristy, Head of Heliopolis research center 13 elmontazh street, sednawy buliding, Heliopolis, Cairo, Egypt.

Email: elshayb1950@yahoo.com

References

1. **Culpetter N, Cole. A, Rowland W.** (eds) effective care in pregnancy and Childbirth, New York Oxford University Press; 1989. Vol. 1.
2. **Berghella V, Odibo OA, TOMS, Rust OA, Althuisius SM, et al.:** Cerclage for short cervix on ultrasonography: meta-analysis of trials using individual patient level data. *Obstet Gyencol* 2005; 106: 181-9.
3. **Fujita K, Nakayama T, Takabatake K, Higuchi T, Fujita J, Maeda M, Fujiawara Hand Mori T:** Administration of thymocytes derived from non-pregnant mice induce an endometrial receptive stage and leukemia inhibitory factor expression in the uterus. *Hum Reprod* 1998; 13: 2888-2894.
4. **Fjuiawara, H, Fukuoka M, Yasuda K, Ueda M, Imai K, Goto Y, Suginami H, Kanzaki H, Maeda M and Mori T.:** cytokines stimulate dipeptidyl peptidase-IV expression on human luteinizing granulosa cells. *J Clin Endocrinol Metab* 1994; 79:1007-1011.
5. **Hashii K, Fujiwara H, Yoshioka S, Hashi K, Fujiwara H, Yoshioka S, Kataoka N, Yamada S, Hirano, T, Mori T et al.:** Peripheral blood mononuclear cells progesterone production by luteal cells derived from pregnant and non-pregnant women: possible involvement of interleukin-4 and 10 in corpus luteum function and differentiation. *Hum Reprod* 1998; 13: 2738-2744.
6. **Nakayama T, Fujiwara H, Maeda M, Inoue T, Yoshioka S, Mori T and Fujii S:** Human peripheral blood mononuclear cells (PBMC) in early pregnancy promote embryo invasion in vitro: HCG enhances the effects of PBMC. *Hum Reprod* 2002; 17: 787-792.
7. **Takabatake K, Fujiwara H, Goto Y, Nakayama T, Hi_chi T, Fujita J, Maeda M and Mori T:** Splenocytes in early pregnancy promote embryo implantation by regulating. endometrial differentiation in mice. *Hum Reprod* 1997; 12: 2102-2107.
8. **Thie M, Fuchs P, Butz S, Sieckmann F, Hoschutzky H, Kemler R and Denker HW:** Adhesiveness of the apical surface of uterine epithelial cells: the rote of junctional complex integrity. *Eur J Cell BioI*1996; 70: 221-232
9. **Mansilla S, Boulaftali Y, Venisse L, Arcas V:** Macrophages are the major source of protease nexin-1. *Arterroscler Thromb Vasc BioI* 2008 Oct. 28(10): 1844-50.
10. **Harger JH:** **Cerclage and cervical insufficiency:** an evidence based analysis. *Obstet Gyencol*, 2002; 100: 1313-1327.
11. **Sakai M, Ishiyama A, Tabata M et al.:** Relationship between cervical mucus interleukin-8 concentration and vaginal bacteria in pregnancy. *Am J Reprod ImmunoI*2004; 52: 106-112
12. **Kleiner DE JR, Stetler-Stevenson WG:** Structural biochemistry and activation of matrix metalloproteases. *Curr Opin Cell BioI*1993; 5: 891-7.
13. **Murphy G:** Matrix metalloproteinases and their inhibitors. *Acta Orthop Scand SuppI*1995; 266: 55-60.
14. **Brew K, Dinakarpandian D, Nagase H:** Tissue inhibitors of metalloproteinases: Evolution, structure and function. *Biochim Biophys Acta* 2000; 1477: 267-83.
15. **Procino G, Carosino M, Marin O, Brunati AM, Contri A, Pinna LA, et al.:** Ser-256 phosphorylation Dynamics of aquaporin 2 during maturation from the ER to the vesicular, compartment in renal cells. *FASEB J* 2003; 17:1886-8.
16. **Hasler U, Nielsen S, Feralle E, Yves-Martin P:** Posttranscriptional control of aquaporin-2 abundance by vasopressin in renal collecting duct principal cells. *Am J Renal Physiol* 2006; 290:F177 -87.
17. **Hildenbrand, Luther Lalitkaumar, Soren Nielsen, Kristina Gemzell:** Expression of aquaporin 2 in human endometrium. *Fertil Steril* 2006; 86: 1452-8.
18. **Rush RW, Isaacs S, McPherson K, et al.:** Randomized controlled trial delivery. *Br*

- Jobstet Gyneco11984; 91: 724-30.
19. **Lazar P, Gueguen S, Dreyfus J, et al.:** Multicentred controlled trial of cervical cerclage in women at moderate risk of preterm delivery. Br J Obstet Gynaecol1984; 91: 731-5.
 20. **Drakeley AJ, Roberts D, Alfirevic Z:** Cervical cerclage for prevention of preterm delivery: meta-analysis of randomized trials. Obstet Gyencol2003; 102: 621-7.
 21. **Shirodkar VN:** A new method of operative treatment for habitual abortions in the second trimester of pregnancy. Antiseptic 1955; 52: 799-800.
 22. **McDonald IA:** Suture of the cervix for inevitable miscarriage. JObstet Gyneco11957; 64: 346-350.
 23. **Quinn M:** Final report of the MRC/RCOG randomized controlled trial of cervical cerclage: Br J Obstet Gyencol 1993; 100: 1154-5.
 24. **Davis G, Berghella V, Talucci M, Wapner RJ:** Patients with a prior failed transvaginal cerclage: A comparison of obstetric outcomes with either transabdominal or transvaginal cerclage. Am J Obstet Gyencol 2000; 193: 836-839.
 25. **Ali Farid M.Ali, Mona Rafik, Laila Farid:** Effect of peripheral blood mononuclear cells on cervix (pregnant either patulous and non patulous, and non pregnant); first prize paper(Golden Medal) presented to the 4th international congress of mediteranian society of reproduction, Roma, Italy 2005.
 26. **Zilianti M, Azuaga A, Calderon F et al.:** Monitoring he effacement of the uterine cervix by transperineal sonography: A new perspective. J illtrasound Med 1995; 14: 719-724.
 27. **Tu CF, Tai HC, Chen CM, Hunag TT et al.:** It improved skin graft survival. Transplant Proc. 2008 Mar, 40(2): 578-80.
 28. **Morton H, Cavanagh AC, Athnanasas Platsis S, et al.:** Early pregnancy factor has immunosppressive and growth factors properties. Reprod Fertil Dev. 1992; 4(4): 411-22.

11/15/2013