MICROSURGICAL VERSUS FIBRIN GLUE ANASTOMOSES OF RABBIT FALLOPIAN TUBE : MICROANATOMICAL DIFFERENCES OF TISSUE HEALING.

SHELAT NIMISH R. ● BOECKX WILLY D. ● BOSMANS LUC.

SUMMARY

From the study of the biopsies, as shown in the figures, we are able to conclude a superior healing of the rabbit fallopian tube with tubal microsurgery. With microsurgery no break in the muscle layers was demonstrable.

Compared to fibrin glue anastomosis of the rabbit fallopian tube, microsurgical anastomosis showed superior muscle apposition, maintained muscle thickness after healing, and no stenosis of the fallopian tube, congenial to normal restoration of its anatomy and physiology, for future fertility and reproduction.

MATERIALS AND METHODS

16 female holland rabbits were chosen for the comparative study. Anaesthesia was given with nitrous oxide, Halothane, with continuous oxygen, after 1 ml Hypnorm injected intramuscularly.

Bilateral flank incisions were to expose the uterotubal junction on each side and each side was dealt with either microsurgery, or fibrin glue anastomoses after dividing the tube at the isthmus.

This was done with a view to expose the anastomoses to the same conditions in the same animal, so the fibrin glue anastomoses on the right, and the microsurgical anastomoses on the left, had the same environment for comparing healing properties.

Microsurgical anastomoses were carried out using 10-0 polyamide monofilament black nylon. A 2 layered anastomoses was done with first the muscular layer, avoiding the mucosa and the second serosal

Dept. of Obst. & Gyn., Surat General Hospital, Surat.
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layer, Eddy (1978) using 8 stitches circumferentially.

Fibrin Glue Scheidel et at (1982) was prepared by adding bovine Aprotinin to a clottable protein complex of fibrinogen, plasma-fibro nectine, factor 13 and plasminogen. Another component was prepared with Thrombin 500 bovine freeze dried added to calcium chloride. Fibrin glue and this component formed an effective sealant glue by quick polymerization. The cut ends of the tubes were brought close together over 0.4 mm diameter polyethylene silastic splint and the tubal lamps (Acland/Winston) tied closely for best possible apposition. After this preparation was complete, 1 drop of fibrin glue (reconstituted) was dropped on the anastomoses through microscopic control and immediately 1 drop of reconstituted thrombin 500 with calcium chroride added Schroeder et al 1987.

After complete drying of the glue on the anastomoses, the tubal clamps were released, and excess glue trimmed off Abdomen was closed in layers. 2 weeks later, Laparotomy was performed to take biopsies of both anastomoses.

Biopsied tissue was fixed in buffered formalin, after parrafin, embedding, histological sections 5 Om thick were cut in 50 levels transverse and longitudinal to the alternate anastomosis. The sections were stained with haematoxylin and eosin Mackay & Khoo 1972.

RESULTS

1) Comparison of open abdomen time.

We compared the time taken for each procedure and found that microsurgery took a mean 15 minutes longer open abdomen time.

Table III : Open abdomen time.

The mean difference of 15 minutes (P > 0.05) with fibrin glue, was less than actually expected, because fibrin glue application required :

1. Careful microsurgical exposure.
2. Clamping both tubes across a splint.
3. Bringing both clamps together with a sutureknot across the clamps.
4. Allowing glue to dry for at least 5 minutes before removing the tubal clamps.
5. Removal of tubal clamps required microsurgical technique of carefully snipping off the excess glue from the clamps, which took some time with every fibrin glue anastomosis.

Table III
Open adbomen time

Animal number.	Fibrub glue anastom.	Microsurgery anastom
1.	65 min	86 min
2.	61 min	80 min
3.	66 min	85 min
4.	63 min	78 min
5.	72 min	80 min
6.	63 min	81 min
7.	62 min	79 min
8.	64 min	77 min
9.	67 min	87 min
10.	66 min	78 min
11.	65 min	76 min
12.	61 min	82 min
13.	73 min	84 min
14.	69 min	78 min
15.	67 min	76 min
16.	65 min	78 min

Thus fibrin glue application, though seemingly easier, required careful handling and more procedures, therefore the time difference between microsurgery and fibrin glue was only 15 min.

2) Comparison : Microscopic tubal apposition.

Microsurgical stitches showed extremely good tissue approximation, with no gap visible after tying the stitchews (Fig.1).

Fig 1 : The muscle fibers are very well approximated and the distribution of fibroblast around satures show that microsurgery successfully approximates the muscle fibers to each other up to a distance of about 300 m bloodvessels. the traction effect of microsutures is clearly demonstratedon this slide where muscles fibers have been pulled together in a ``horse shoe" disposition which indicates useful traction for restoration of the anastomosis.

But with fibrin glue anastomoses in absence of stitches, there was lack of appropriate apposition of the tubal musculature despite proper efforts to appose the anastomoses over a polyethylene splint and with tubal clamps tied closely together. Sojo et al 1983. This was visible through the microscope as a 'gap', (Fig.2)

Fig. 2 : The gap in the muscle is filled with fibrin glue. The epithelium has regenerated well in these two weeks but is still cuboidal with round or oblong nuclei at the same level. Note the pseudo-stratified columnar epithelium in the vicinity.

It was decided to evaluate the healing process with the two diferent techniques and, therefore biopsies were carried out from respective animals after 2 weeks.

There were no major adhesions on the second laparotomy; except in the first animal, we had adhesions. The adhesions were scored according to Winston 1975.

Table IV : Adhesions formation.

There was no significant difference (X, $P \geq 0.05$) between adhesions following fibrin glue or microsurgical anastomoses, at second laparotomy for biopsy performed, 2 weeks after surgery.Sorensen et al 1987.

Table IV
Adhesions formation

	Microsurgery	Fibrin glue
-	15	14
+	0	1
++	1	1
+++	0	0

- : no adhesions
+ : mild adhesions
++ : moderate adhesions
+++ : severe adhesions

3) Histology :

In the surgical group at the site of the anastomosis no break in the circular, and longitudinal muscle layers of the tube was demonstrable. There was no stenosis macroscopically at the time of biopsy at 2 weeks post surgery.

The preudostratified, ciliated columnar epithelim, regenerated normally, with very little foreign body reaction.

With the fibrin glue anastomosis, however the muscle of the tube showed an area of few cells at the site of the anastomosis with some atrophy after two weeks, macroscopically this was visible as a stenosis at review laparotomy for

Table V
Histology
(MS = Microsurgery, FG = Fibrin Glue)

	Muscle apposition				Muscel thickness			Lumen Stenosis		
	E	G	F	P	F	P	L	N	P	C
MS	12	3	1	0	15	1	0	16	0	0
FG	0	1	2	13	1	1	14	3	13	0

Muscle apposition
E : Excellent 100% - 75%
G : Good 75% - 50%
F : Fair 50% - 25%
P : Poor 25% - 0%
Muscle thickness
F : full thickness 75% - 100%
P : partial thickness 50% - 70%
L : low thickness < 50%
Lumen stenosis
N : no stenosis
P : partial stenosis
C : complete stenosis

biopsy.

This is the area where the muscle fibres, after having been cut, retracted away from each other, with fibrin glue anastomosis. There was no traction effect on either side for effective apposition.

With the healing achieved with microsurgery where muscles are brought together in close approximation, with the microsutures (Fig.1). The muscle fibres are very well approximated and the distribution of fibroblast around sutures shows that microsurgery successfully approximates the muscle fibres to each other upto a distance of about 300 O. without impairing its blood supply as indicated by new outgrowing bloodvessels.

The regeneration of the mucosa has been the same as with microsurgery as evident from the histology with initial cuboidal, non-ciliated epithelium, replaced by the pseudostratified, ciliated columnar epithelium after 2 weeks in all cases. Eddy et al (1978).

From the study of the biopsies, we have observed satisfactory healing with microsurgery of the rabbit fallopian tube. However with fibrin glue anastomosis, histology showed poor continuity of the muscle at the site of the anastomosis despite no difference in mucosal regeneration.

Table V : Histology.

As shown in table V there was a statistically significant difference between the muscle apposition in the Microsurgical group and the Fibrin blue group (X, O < 0.001).

DISCUSSION

We believe that this should affect the ovum transfer with fibrin glue anastomosis because of the muscle retraction at the site and contractile properties across the anastomosis may be impaired. For a normal physiology to propel the non motile ovum, not only the cilia of the mucosa are important, but equally important is the tubal motility of the muscle itself and tubal peristalsis especially in the isthmus and ampullo-isthmic junctions. Detailed electromyografic studies Archer et al 1979 with fibrin glue anastomosis are required for further analysis.

CONCLUSION

New techniques have been tried to replace Microsurgical principles, with recent advances. The use of Fibrin Glue, substituting this tissue sealant for microsutares has not proven to be superior than microsurgery in this study. In fact the first study showed statistically significant reduced reproductive outcome, the genesis of which was reflected in the second study of detailed histology of the biospsies obtained from anastomotic site. Electromyographic studies are in progress at the center for Microsurgery, University of Leuven, which would additionally provide an insight into the physiological differences between Microsurgical and Fibrin Glue anastomoses of the rabbit Fallopian tube.

Till further research conclusively helps in proving advantages with fibrin glue, microsurgery still retains its vital place in Fallopian tube reconstruction, and as yet no short cuts exist in effectively reconstructing this vital organ of the mammalian species, where life begins and conception occurs.

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