Prenatal Cleft Lip and Maxillary Alveolar Defect Repair in a 2-Step Fetal Lamb Model

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Purpose: As there is no satisfying animal model simulating the complex cleft lip and palate anatomy in a standardized defect on one hand, and comprising the possibilities for extensive surgical procedures on the other hand, an improved fetal lamb model for cleft surgery was developed.

Materials and Methods: Prior to the main study with 16 animals, a pilot study with 4 lambs was conducted. In the pilot study, the unilateral defect was induced at 75 days of gestation. Within 22 days the defect was repaired in 3 lambs; 1 lamb remained unoperated. Disappointing results from the pilot study led to an earlier defect induction (60 to 64 days of gestation) and earlier repair (71 to 84 days) in the main study with 16 lambs. The subsequent delayed repair of the defect was carried out using a Tennison-Randall technique in 10 lambs. In 4 lambs the defect was repaired postnatal, using the same technique. Two lambs had to be excluded from the study. After being euthanized, all animals were investigated macro- and microscopically.

Results: According to our criteria, the esthetic results ranged from satisfactory to good. Cutis and mucosa showed a full recovery whereas subcutis and the orbicularis oris muscle showed healing with scar formation. On average the operated lips were 9% shorter and were also thinner than the contralateral control side.

Conclusions: In this study, the results of the closure of a standardized lip and maxillary alveolar defect in several stages of gestation were documented. Early intervention led to better esthetic results, but increased the risk of abortion by 25%. There was no prevention of scarring in subcutaneous and muscle tissue. Because there was no alignment of the orbicularis oris muscle, the goal of a functional perfect result was not achieved.

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Due to recent advances in the development and improvement of high-resolution ultrasound, early diagnosis of congenital anomalies such as cleft lip and palate (CL-P) is now a reality. As early fetal wound healing can result in scarless tissue regeneration, fetal surgery may be a treatment option in the future.¹

Nowadays in utero surgical interventions are becoming safer and safer for the mother as well as for the fetus. In this way fetal surgery may become a treatment option for non life-threatening pathologies, such as CLP, in the near future. The potential benefits of an early fetal intervention could dramatically decrease the number of postnatal reconstructive procedures and so reduce the detrimental scar-induced effects of postnatal CLP repair.²

This study was conducted to investigate the technical problems and drawbacks of open cleft lip and

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maxillary alveolar defect repair in a fetal animal model. As there was no sufficient cleft model simulating the complex cleft anatomy on one hand, and comprising the possibilities for extensive surgical procedures on the other hand, an improved lamb model was developed in this trial.2-10

Materials and Methods

As fetal lambs do not form isolated cleft lips in response to teratogens,11 a cleft lip as well as a maxillary alveolar defect had to be induced surgically. Prior to the main study, a pilot study with 4 lambs was performed. Disappointing results from the pilot study led to an earlier defect induction and repair in the main study (Fig 1). All experiments in this study were approved by the committee for ethics and by the committee for animal care and use of the Rheinische Friedrich-Wilhelms-University of Bonn.

MAIN STUDY

After hysterotomy (Fig 2), unilateral cleft lip and maxillary alveolar defects were induced in 16 fetal lambs at 62 to 64 days of gestation: A rhombic full thickness defect was induced by cold knife surgery in the lateral upper lip (Figs 3A,B). A cuneiform alveolar defect was added with a pair of microsurgical scissors. To avoid immediate closure, the borders of the defect were assured by sutures (Fig 3C). Each lamb was photodocumented (Cyber-shot 3.3 Mega pixels; Sony Deutschland GmbH, Köln, Germany) before and after the intervention. The lamb was relocated in the womb and the amniotic fluid (which had been sampled and kept on temperature) with 500 mg ampicillin (Unacid; Pfizer, Karlsruhe, Germany) added to it.
was reinstilled in the uterus. This procedure was followed by wound closure of uterus and abdominal wall in layers.

Eleven to 14 days after the first operation the defects (Fig 4) were repaired in 10 of the lambs. A control group of 4 lambs was intended to receive cleft repair postnatally. The defect closure was carried out using Tennison-Randall technique (Fig 5) in the prenatal as well as in the postnatal group.

Five days before birth (gestation is approximately 145 days) the ewes were separated from the flock. The lambs were delivered naturally or by section. After delivery, all lambs that had fetal surgery received 6 mg/kg xylazine intramuscular (Vexylan, Ceva Santé Animale, Brussels, Belgium) for sedation. The results of defect repair were assessed macroscopically for alignment of the vermilion border, vertical height in comparison to the contralateral side, wound dehis-
cence, midline shift, visible scars, axis of the nostrils, and persistency of the maxillary alveolar defect. Ten minutes after sedation the lambs were euthanized by injection of 0.5 mL T61/kg (Intervet, Unterschleißheim, Germany). When death was ascertained, the upper jaws were removed and preserved in formalin 4% for histology.

Three of the 4 lambs in the control group were operated on from 14 to 21 days postnatal. Eight weeks after defect repair those lambs were euthanized and the results of surgery were appraised as described above.

After formalin fixation, the jaws were decalcified for 8 weeks in a solution of 100 g EDTA (Calbiochem, Darmstadt, Germany), 34 g TRIS (ICN Biochemicals, Cleveland, OH) in 1,000 mL distilled water. The decalcified tissue samples were embedded in paraffin and cut in 10 μm sections always including the region of the repaired lip and alveolar defect as well as the contralateral side; the section plane was aligned to the palate. The histology of the affected side was compared with the contralateral side of each animal. Tissues for light microscopic evaluation were either stained with hematoxylin and eosin, elastica, alcin blue, or periodic acid-Schiff. Immunohistochemistry was performed for type I and type III collagen. The results were photodocumented (Kodak EPY-Ektachrom 64T, Kodak GmbH, Stuttgart, Germany).

FIGURE 4. The defect as it appeared at the time of repair immediately after removing the Prolene stitches, just before defect repair.


Results

On average the cleft was induced at 62 days of gestation and repaired at 76 days. In the main group \((n = 12)\), 10 lambs received fetal defect repair, and 5 lambs were delivered preterm (survival rate: 50%). From those 5 preterm lambs, 2 had to be excluded from the macro- and microscopic evaluation.

In the control group \((n = 4)\), 1 lamb had to be excluded from the study, so the survival rate was 75%. On average the cleft was induced at 64 days of gestation and repaired 14 to 21 days after delivery (Fig 1).

MACROSCOPIC EVALUATION

There were no dehiscent wounds. In general, there was an exact alignment of the vermillion border. The cranial traction of the operated lip was less than in the pilot study; nevertheless the average height of the operated side was 9% shorter than the contralateral lip. There was a slight midline shift towards the operated side in 4 lambs; in the other 4 lambs, there was no midline shift at all. The operated nostril was deformed and smaller in 1 of the lambs. The alveolar maxillary defect was still visible in 2 lambs; in 6 lambs there was no evidence of the defect. In summary the results were judged as good in 3 (Fig 6A) and as satisfactory in 4 lambs. Only 1 lamb showed an unsatisfactory result (Fig 6B).

In the control group, 1 dehiscent wound after postnatal defect repair was seen. The esthetic result in this case was judged as unsatisfactory. In the 2 remaining lambs, the results concerning alignment of the vermillion border, vertical height of the lip, midline shift, visible scars, axis of nostril, and persistent maxillary alveolar defect were judged as good.

MICROSCOPIC EVALUATION

Epidermis and mucosa showed a full recovery. The repaired side was thinner than the contralateral side. The presence of connective tissue fibers indicated scar formation. The number of hair follicles in the defect region was reduced, the salivary glands were degenerated, and their excretory ducts were dilated. There was no alignment of the orbicularis oris muscle in the defect (Fig 7). Subepithelially, there was only weak type I collagen, but distinct type III collagen staining was detectable.

Discussion

The first studies concerning fetal surgery in animal models date back to the beginning of the former century.\(^1\) Since then a number of surgical procedures have been established in different animal models. Among them a number of authors described the induction and repair of cleft lip and palate defects in mice\(^1\) rabbits,\(^1\) apes,\(^1\) as well as in sheep.\(^2\)


The advantages of small animals (mice, rats, rabbits) are lower costs and a higher number of fetuses per animal. Disadvantages are the complicated surgery because of the midget anatomy of the fetuses and the short period of gestation. The small anatomy does not allow complex surgical procedures such as Tennison-Randall cleft repair. The short gravidity increases the risk of scheduling the intervention late in
time, thus missing the point of time when fetal wound healing is still possible.\textsuperscript{22,23}

Bigger animals (sheep, goats, apes) provide a more realistic anatomy for the application of complex surgical techniques. Because all animals had to undergo 2 operations (cleft induction and cleft repair), the ewes’ long gestational period of approximately 145 days was of major importance. Furthermore ewes have a remarkable resistance to premature delivery.\textsuperscript{2-5,7-10} As the fetal lamb does not form clefts in response to teratogens as cyclophosphamid, phenytoin 8, or anabasin 24, the CL-P defect had to be induced surgically. Although creating a cleft by teratogens better mimic reality, the artificial defect can be standardized and is therefore reproducible.

Consistent with the literature\textsuperscript{10} the mortality of the fetal lambs in this study was dependent on the point of time the operation was accomplished. As premature wound healing becomes more and more adult-like during fetal development\textsuperscript{2,15,24} the defect repair was performed on average at 76 days of gestation thus increasing mortality from 25\% in the pilot study to 50\% in the main study. At this point of time 50\% to 55\% of the gravidity is over. In the

\begin{figure}
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\includegraphics[width=\textwidth]{figure7.png}
\caption{Documentation of the histologic architecture. For a better orientation, oral, extraoral, and repaired defect region are labeled. A, The defect region is thinner than the contralateral side and B, the number of hair follicles is reduced. C, The salivary glands are degenerated (XX) and their excretory ducts are dilated (YY). D, Degeneration of muscle fibers (Y) and presence of connective tissue fibers (YY) indicate scar formation (circled area). Original magnification: A, B, $\times$25; C, D, $\times$100.}
\end{figure}

human fetus, the transition from fetal into adult wound healing is at presumably 22 weeks of gestation, when 55% of gravidity is over. This means that an intervention should be carried out before those 22 weeks of gestation, which is nowadays still high at risk. Recent advances in fetal endoscopic surgery might decrease the perisurgical mortality. Nevertheless there are still a lot of unsolved risks, among them premature rupture of membranes being the most important one.

In prior studies, cleft induction and repair were performed in a single operation. Macroscopic and microscopic results were close to a full recovery in all tissue layers. Nevertheless, the complex deforming mechanism of a cleft on its anatomical surrounding was not simulated. The need for defect reconstruction techniques (especially elongation of the lip) arising from the anatomical situation was not respected. In surgical induced clefts, the continuity of the orbicularis oris muscle is interrupted and there is no insertion of the muscle fibers in the cleft region, thus affecting the size and configuration of the defect as seen in congenital clefts.

In this study, the technique of cleft induction assured a dehiscent cleft with formation of epithelium in the border area of the lesion and a deformation of the affected nostril (Figs 2-7). To our knowledge this is the most realistic and reproducible surgically induced cleft model in bigger animals at the moment.

A not surgically induced, congenital cleft palate (without cleft lip) model was described by Weinzweig et al in goats. A not surgically but congenital cleft lip and palate induction is still not possible in bigger animals.

Cleft repair in human beings achieves an exact horizontal alignment and vertical elongation of the cleft lip segments by using different techniques, such as Veaü, Millard, Pfeifer, or Tennison-Randall. Those techniques have not yet been used for fetal surgery. In this study, the Tennison-Randall technique was used for cleft repair in a fetal lamb model. As described, the results were not really successful. This probably has to do with surgical pitfalls. Because a fetal lip has the consistency of an English plum pudding, it was not possible to dissect the orbicularis oris muscle and apply sutures. Therefore the defect repair of all layers (skin, muscle, and mucosa) as postulated by Veaü could not be performed. As a consequence, the light microscopic evaluation of the stained tissues showed no alignment of the orbicularis oris muscle.

In the repaired defect tissue, there was evidence of scar tissue with degeneration of the salivary glands and the hair follicles (Fig 4). Although there was a full recovery of cutis and mucosa, there was clearly scarring of the subcutis and the muscles. Therefore, fetal wound healing seems to be organ-specific. In contrast to skin and mucosa, lesions in fetal nerve tissue, stomach, trachea, myocardium, and diaphragm do not heal unscarred as well as the repaired fetal cleft lip and maxillary alveolar defects in this study which did not heal scarless. Because the macro- and microscopic evaluation of the cleft repair was conducted immediately after birth, it is likely that initially satisfying results will not improve during or after the growth spurt. This would finally result in 1 or more secondary operations. In this way the potential benefits of fetal cleft repair would be eliminated.

At the present time it does not seem to make sense to hazard the consequences of the risk of prenatal operations in any other case except for those fulfilling the 1991 criteria of the International Fetal Medicine and Surgery Society. The ambition of future studies must be the development of much more realistic animal models to understand and solve the problems associated with fetal cleft surgery. Another, probably better way to improve the results of cleft surgery is to understand and learn how to make adult wounds more fetal-like.

References