Lupine Research

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Introduction

- Over 500 species of annual or perennial lupines in North America
- Over 150 quinolizidine/piperidine alkaloids have been identified
- “Crooked calf syndrome” geographically widespread and economically important
- Other plants cause the same syndrome
  - Poison-hemlock
  - *Nicotiana* spp.

Factors Affecting Incidence of "Crooked Calf Syndrome"

- Environmental
  - Moisture previous seasons
  - Population cycles
  - Other available forage and quality
- Lupine Species
  - Taxonomy
  - Chemical composition

Conium, *Nicotiana*, and *Lupinus* spp.

Lupines
(*Lupinus* spp.)

Poison-
hemlock
(*Conium*
maculatum)

Nicotiana spp.
(*Nicotiana tabacum*,
N. glauca)

Historical Aspects

Poison-hemlock:
Cleft palate/
MCC pigs

Lupine-induced
“Crooked Calf
Syndrome”

Nicotiana spp.:
Cleft palate/
MCC pigs

Poison-hemlock
Conium maculatum

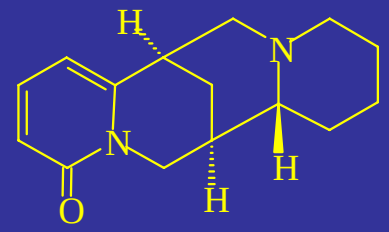
Coniine (11.4)

Nicotiana spp.

Anabasine (1.6)

Lupine

Ammodendrine
(134.4)



Anagyrine (7.2)

Lupines

Anagyrine

Agriculture

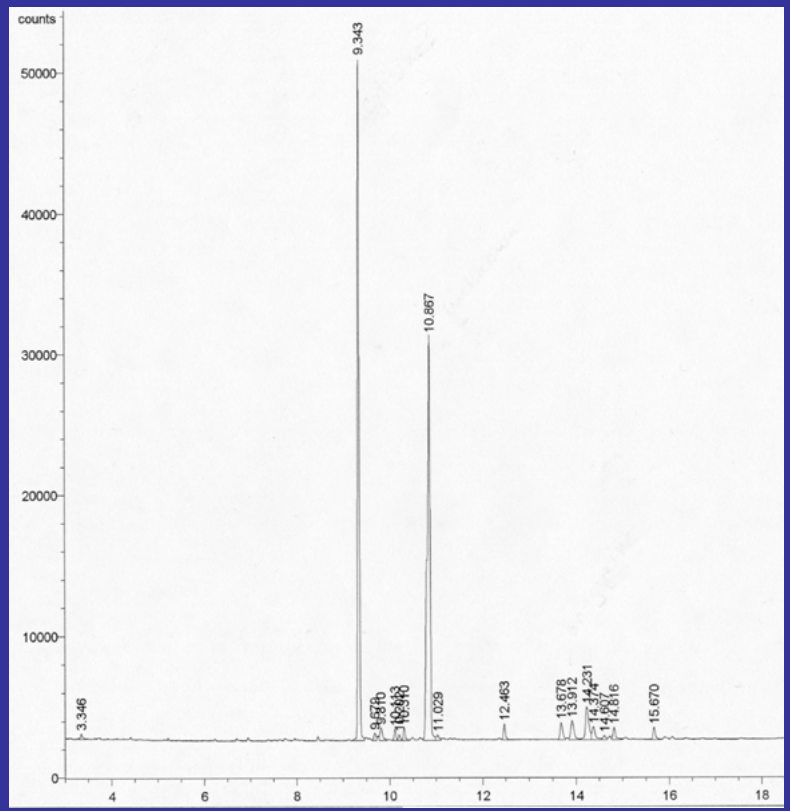
Weed causes epidemic of mutant calves

WASHTUCNA, Wash. (AP) — Spring calving season is off to a troubled start in Adams County, where an epidemic of deformities in newborns has left ranchers desperate.

About one of every five calves born in recent weeks has been put to death with a bullet to the head.

Ranchers say it's the best way to end the misery of animals born with crooked calf syndrome. The malady leaves calves facing an almost certain death from starvation or being eaten by coyotes.

Chromatograms



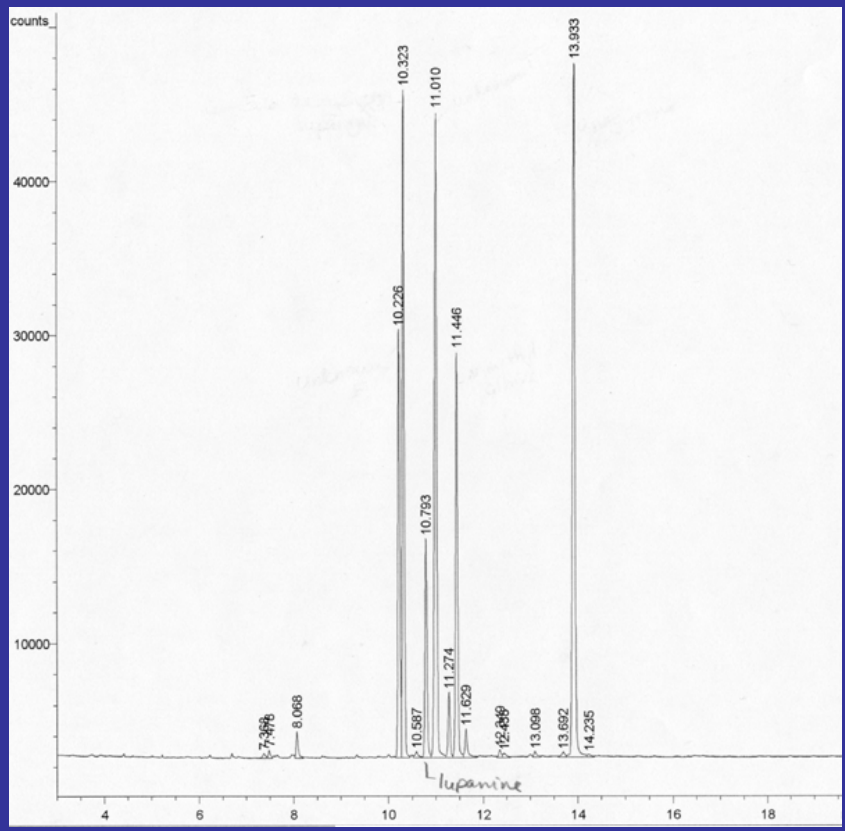


Table 1. Total and individual alkaloid concentrations (mg alkaloid/100 mg dry plant) in *L. sulphureus* at different stages of plant growth.

Plant stage	Date	Total	J	K	C	L	E	% of E[1]
Pre bud	4-02-93	1.55	TR	-	0.43	TR	1.11	70%
Early bud	4-16-93	1.25	0.06	0.26	0.21	TR	0.73	58%
Early bloom	4-28-93	1.43	0.08	0.05	0.35	0.03	0.91	64%
Full bloom	5-04-93	0.98	0.10	0.15	0.13	0.01	0.59	60%
Late flower/ early pod	6-15-93	0.80	0.08	0.09	0.28	0.03	0.30	36%
Mature 75% Seed shatter	6-23-93	0.24	0.06	0.02	0.05	-	0.13	54%
Flowers	5-04-93	1.68	0.12	0.09	0.15	0.02	1.30	78%
Leaves	5-04-93	1.23	0.13	0.12	0.22	0.02	0.73	59%
Stems	5-04-93	0.24	0.06	0.02	0.04	-	0.14	61%
Seed	6-23-93	4.17	0.68	0.23	1.26	0.02	1.84	44%

[1] = % of E in the total alkaloid content
E = The teratogen anagyrine
J = 5, 6-Dehydrolupanine
K = Lamprolobine
C = Lupanine
L = Unidentified Alkaloid (represented by 1 chromatographic peak)

Note: Letters represent peaks on the chromatogram.

Table 2. Total and individual alkaloid concentrations (mg alkaloid/100 mg dry plant) in *L. leucophyllus* at various stages of plant growth. Note: no anagyrine was detected.

Plant stage	Date	Total	A	F	H	I
Early growth	4-02-93	1.30	0.11	0.17	0.26	0.75
Pre bloom	4-28-93	1.44	0.17	0.32	0.15	0.75
Mid-late bloom	6-23-93	0.94	0.11	0.25	0.05	0.53
Late bloom/ early pod	6-23-93	0.89	0.15	0.27	0.04	0.44
Mature seed heads	7-09-93	1.42	0.04	0.38	TR	1.00
Seedlings	7-09-93	1.31	0.40	0.24	0.07	0.59

A = Tetrahydrorhombifoline
F = 13-hydroxylupanine
H = 13-angeloyloxylupanine
I = Unidentified Alkaloids (represents a total of 4 chromatographic peaks)

Note: Letters represent peaks on the chromatogram.

Table 3. Total and individual alkaloid concentrations (mg alkaloid/ 100 mg dry plant of *L.leucophyllus* (A) and *L. sulphureus* (B) collected on 6-2-93 in different pastures, and before and after herbicide treatment for *L. sulphureus* (C). *L. Sulphureus* in the east pasture had senesced and was not analyzed. Note: No anagyrine was detected in *L. leucophyllus*.

L. leucophyllus (A)

Pasture	Total	A	F	H	L
West	1.38	0.20	0.43	0.03	0.72
Top	0.86	0.05	0.34	TR	0.42
East	0.51	0.06	0.19	TR	0.25

L. sulphureus (B)

Pasture	Total	J	K	C	L	E
West	1.08	0.08	0.07	0.54	0.07	0.27
Top	1.25	0.08	0.27	0.25	0.02	0.59

L. sulphureus (flower stage) (C)

	Total	J	K	C	L	E	% anagyrine in total
Untreated	0.73	0.09	0.08	0.13	TR	0.44	61
Treated	0.95	0.06	-	0.39	0.02	0.37	43

A = Tetrahydrorhombifoline
C = Lupanine
E = Anagyrine
F = 13-Hydroxylupanine
H = 13-Angeloyloxylupanine
J = 5, 6-dehydrolupanine
K = Lamprolobine
L = Unidentified Alkaloids (represents a total of 4 chromatographic peaks for *L. leucophyllus* and 1 peak for *L. sulphureus*)

Note: Letters represent peaks on the chromatogram.

Table x.2. Alkaloid daily dosage (mg/kg body weight) and outcome of pregnancy in cows fed *L. formosus* and *L. arbustus*.

Cow#	Group#	Treatment	T	A	B	C	Results
5991	1	87-3	122	25	8.6	28.8	Clinical signs mod-sev, calf normal
6038	1	87-3	133	27	10	31.6	Clinical signs mod, CP
6041	1	89-1	84	27	11.5	17.7	No effects, calf normal
6008	2	94-1	42	28	----	----	No effects, ED/R
6025	2	94-1	42	28	----	----	No effects, BK/CF
6030	3	ctr	----	----	----	----	Calf normal
6026	3	ctr	----	----	----	----	Calf normal

T=Total alkaloid daily dosage (mg/kg)
A=Daily ammodendrine dosage
B=Daily *N*-methyl ammodendrine dosage
C=Daily *N*-acetyl hystrine dosage
CP=Cleft palate
ED/R=Embryonic death/resorption
BK/CF=Bucked knees/carpal flexure
87-3 and 89-1= *L. formosus*; 94-4= *L. arbustus*; ctr= water control

Alkaloid Dosages

L. formosus 0.8 mg/gm ammodendrine = 10.6 mg/kg BW

L. arbustus 0.4 mg/gm ammodendrine = 5.3 mg/kg BW

L. caudatus 3.4 mg/gm anagyrine = 43.8 mg/kg BW

Teratogenic levels in cow: 2.1 - 2.9 mg/kg ammodendrine
6.5 - 11.9 mg/kg anagyrine

Defects/Susceptible Gestational Periods

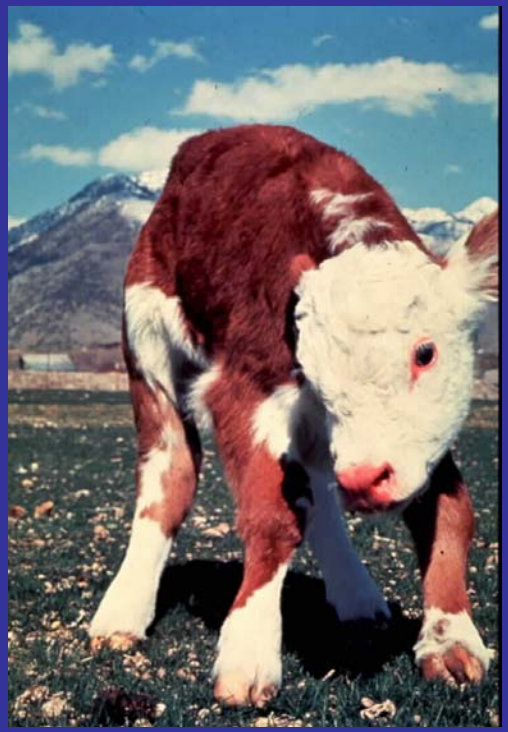
- Multiple Congenital Contractures 40-70 days
  - Arthrogryposis
  - Scoliosis
  - Torticollis
  - Kyphosis
  - Lordosis
- Cleft Palate 40-50 days

Teratogenic Effects

Arthrogryposis

Scoliosis

Teratogenic Effects-*cont.*



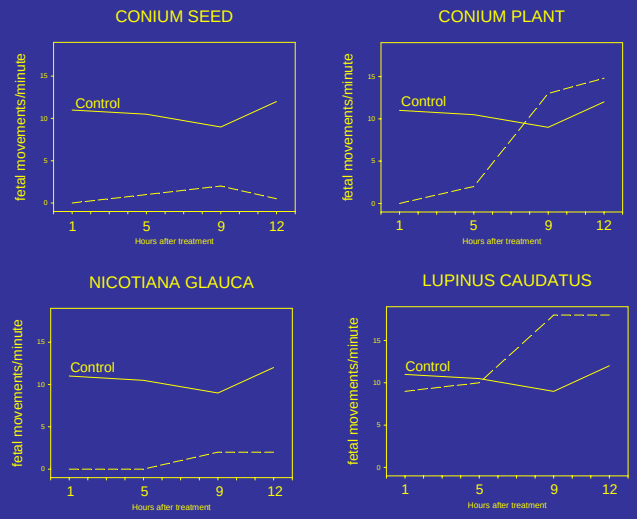
Torticollis

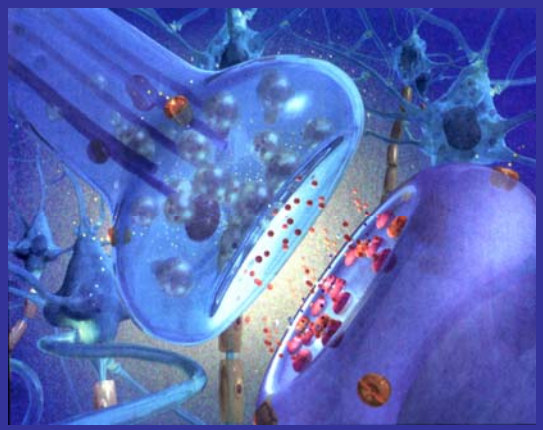
Kyphosis

Cleft Palate

Mechanism of Action

Alkaloid-induced reduction in fetal movement

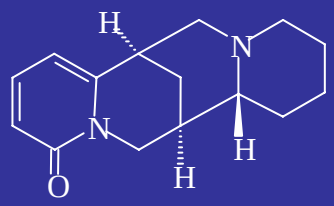




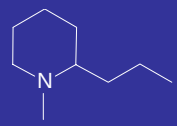
Blocks neuromuscular junction

Mechanism of Action

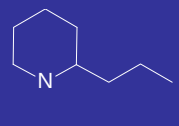
Toxic and Teratogenic Piperidine Alkaloids



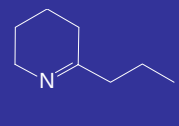
Anagyrine



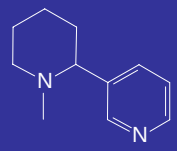
N-methylconiine
20.54 mg/kg
19.12 - 22.08



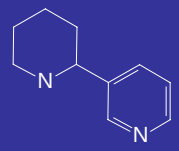
Coniine
11.41 mg/kg
9.55 - 13.64



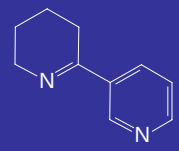
γ-Coniceine
2.52 mg/kg
2.33 - 2.73



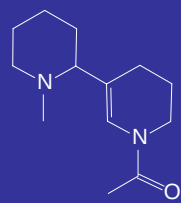
N-methylanabasine
12.46 mg/kg
10.06 - 15.43



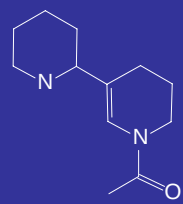
Anabasine
1.62 mg/kg
1.5 - 1.75



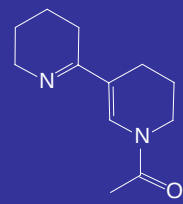
Anabaseine ?
1.05 mg/kg
1.048 - 1.057



N-methylammodendrine
110.68 mg/kg
103.19 - 118.7



Ammodendrine
134.38 mg/kg
128.34 - 140.71



N-acetylhystrine (80%)
29.7 mg/kg
27.81 - 31.73

Management Recommendations

1. History of lupine poisoning
   a) Survey pastures/rangelands
   b) Historical losses

2. Identify lupine species or other poisonous plants
   a) Taxonomy
   b) Chemical composition

Management Recommendations

3. Management changes
   a) Intermittent grazing (clean vs. infested pastures)
   b) Changing breeding program (avoid lupine during susceptible gestational period)
   c) Intense management (cull lupine eaters or move them to clean pastures)

Management Recommendations

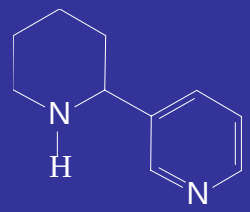
4. Herbicide control
   a) 2,4-D at 2 lb/ai/ac
   b) 2,4-D + Dicamba at 1:0.5 lb ai/ac
   c) Triclopyr at 0.5-1.5 lb ai/ac
   d) Escort (metsulfuron) at 0.5-2 oz/ac

5. Beneficial use of lupine pastures
   a) Lupine is good feed (12-18% protein, 30% + in seeds)
   b) Graze steers or open heifers
   c) Graze infested pastures after seed pods shatter

Biomedical Applications

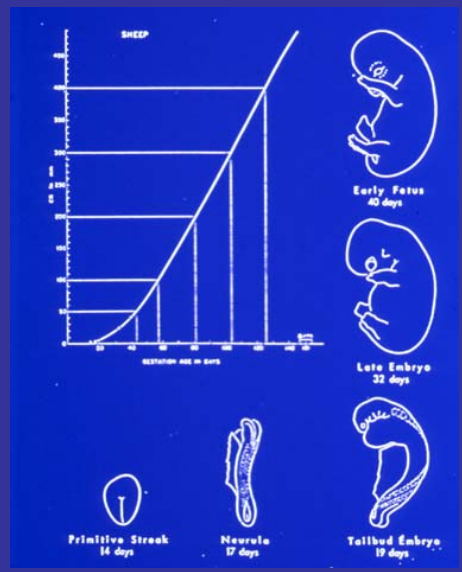
- Study the etiology of cleft palate in humans
- Develop *in utero* surgical repair techniques
- Develop techniques and procedures to improve treatment of the human anomaly

Goat Model



Anabasine

- Developed to study mechanism of action of lupine-induced Crooked Calf Disease
- Utilizes *Nicotiana glauca* (anabasine)
- Cleft palate induced during days 32-41 of gestation



Incidence of Cleft Palate in Humans

- Cleft lip or cleft lip and palate occur in about 1 out of every 1000 live births over all groups
  - In Asian groups, the incidence is 1.7 per 1000
  - Among certain American Indian groups, the incidence is 3.6 per 1000
- Depending on the severity of the deformity, between 4 and 16 surgeries are required to repair the cleft lip and/or palate

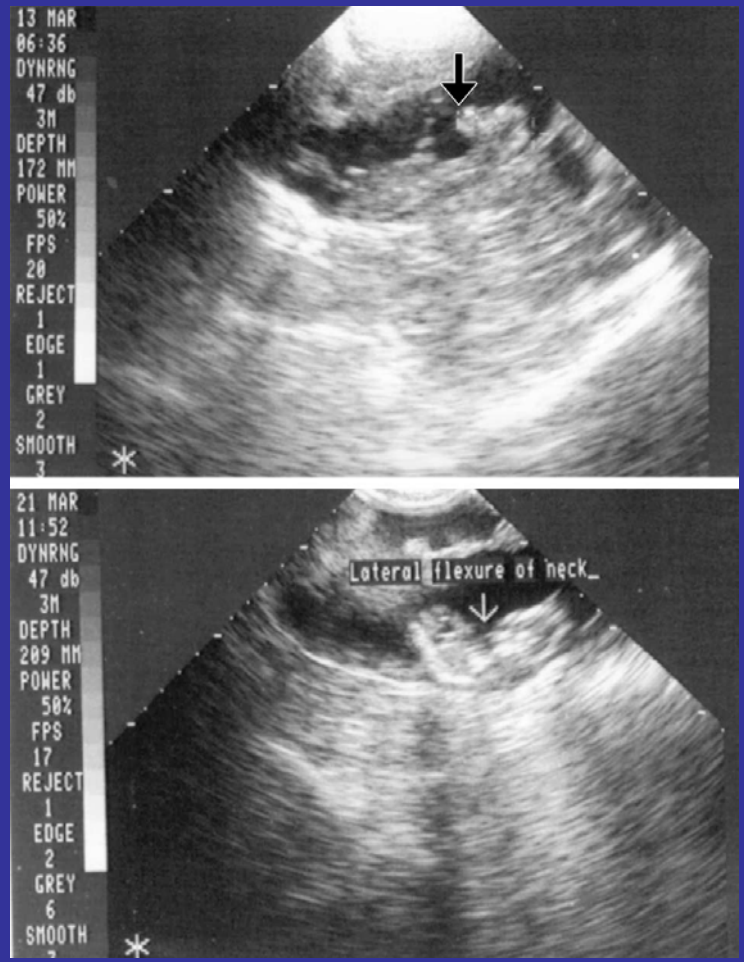
Goal of this Research

- Improve treatment for cleft palates in humans

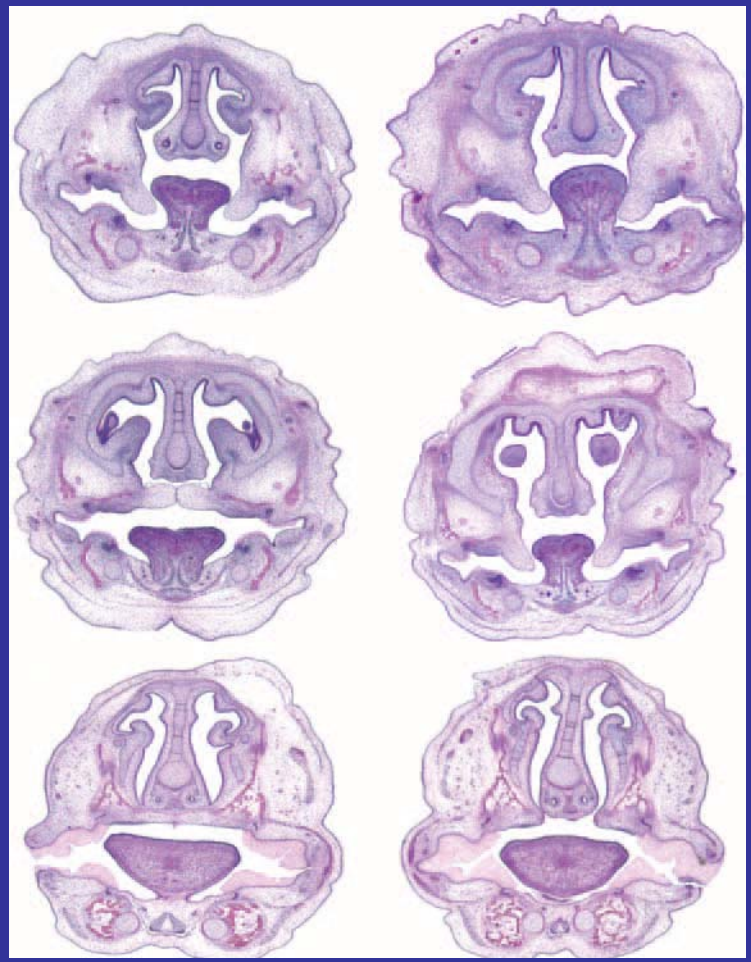
  "Children born with cleft palate often undergo a series of operations to correct the ensuing deformities, only the first of which is the actual palate repair at the age of 6-12 months. For many children, speech remains a major problem as well as craniofacial development. Our goal, of course, is to eliminate the need for any of these reconstructive procedures by performing the cleft palate repair *in utero*. Never, more than now, in the age of fetal surgery has this been a real possibility. Despite this, what is truly exciting is that we now have a congenital goat model of cleft palate as well as the model of *in utero* repair."

Dr. Jeff Weinzweig, 1999
Chairman, Department of Plastic & Reconstructive Surgery
Lahey Clinic Medical Center, Burlington, MA

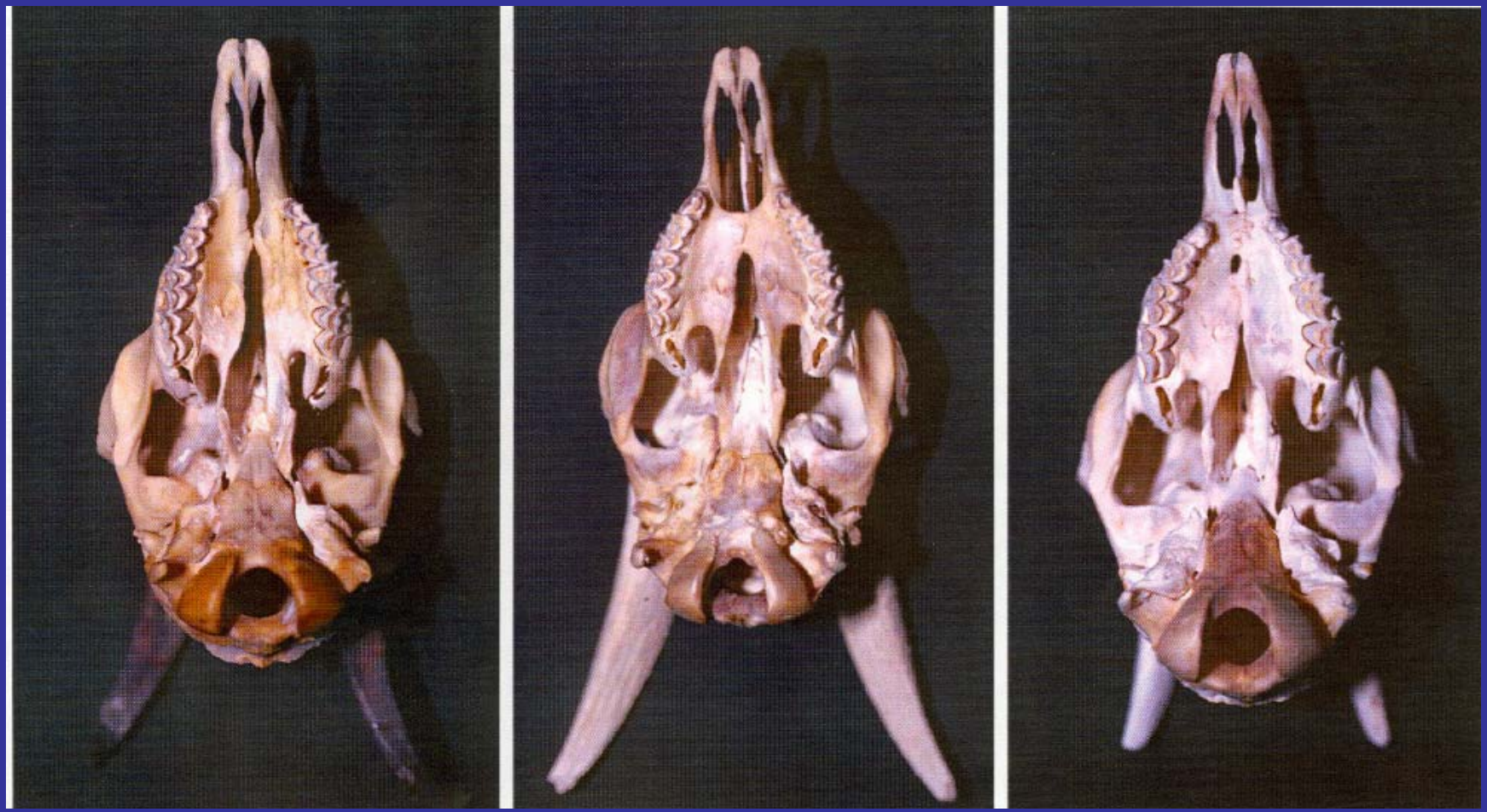
Neck flexure



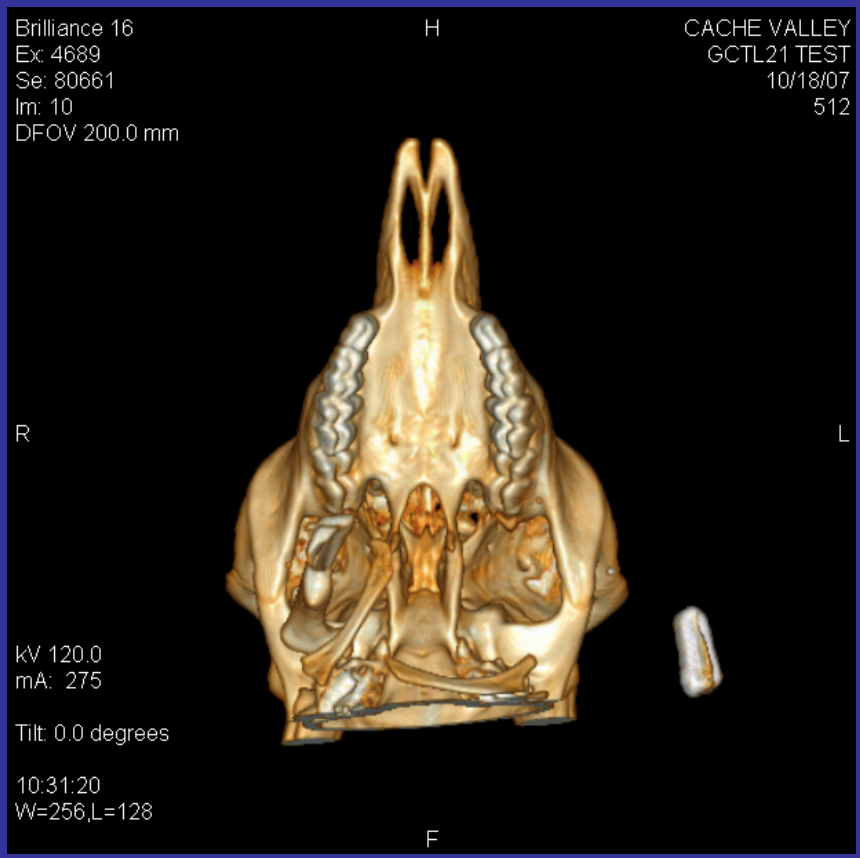
Coronal histology views



Bony palate from repaired soft tissue palate



CT 3D views


Brilliance 16
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Se: 80661
Im: 10
DFOV 200.0 mm
H
CACHE VALLEY
GCTL21 TEST
10/18/07
512
R
L
kV 120.0
mA: 275
Tilt: 0.0 degrees
10:31:20
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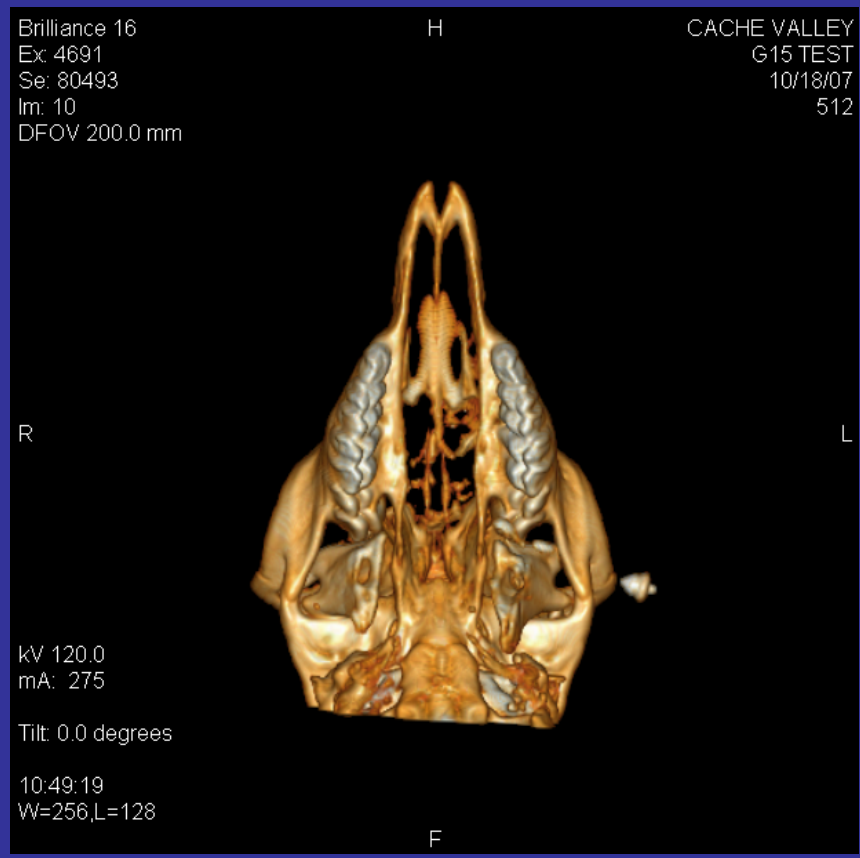
F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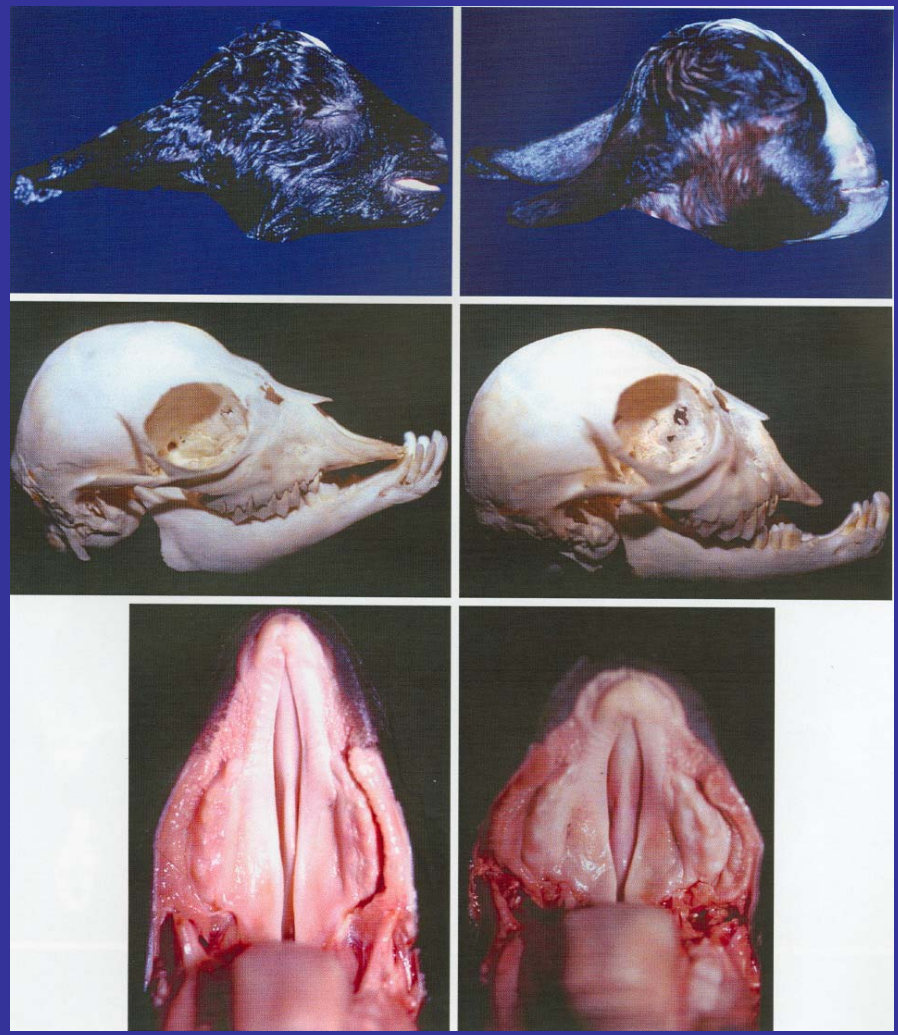
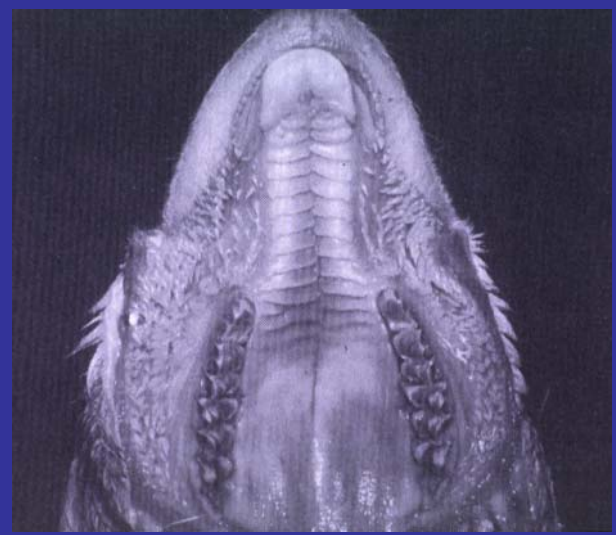
Maxillary hypoplasia

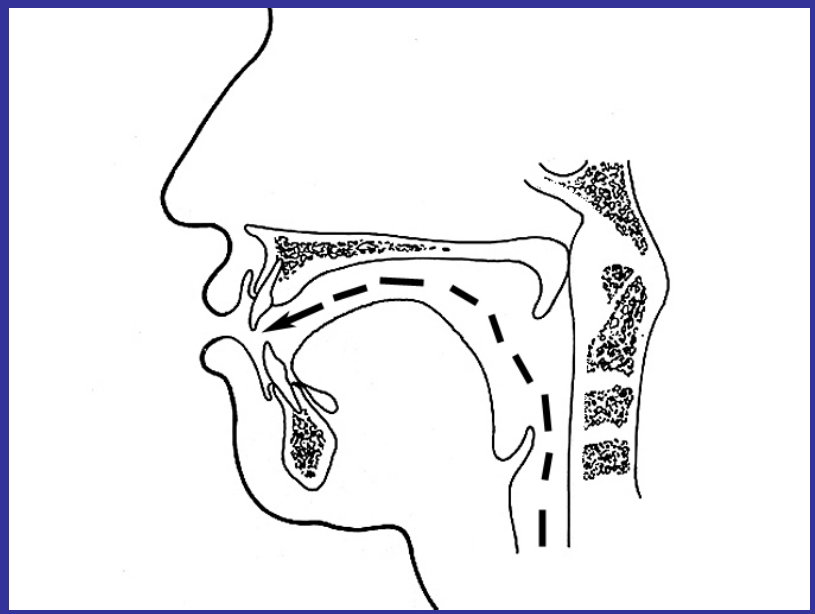


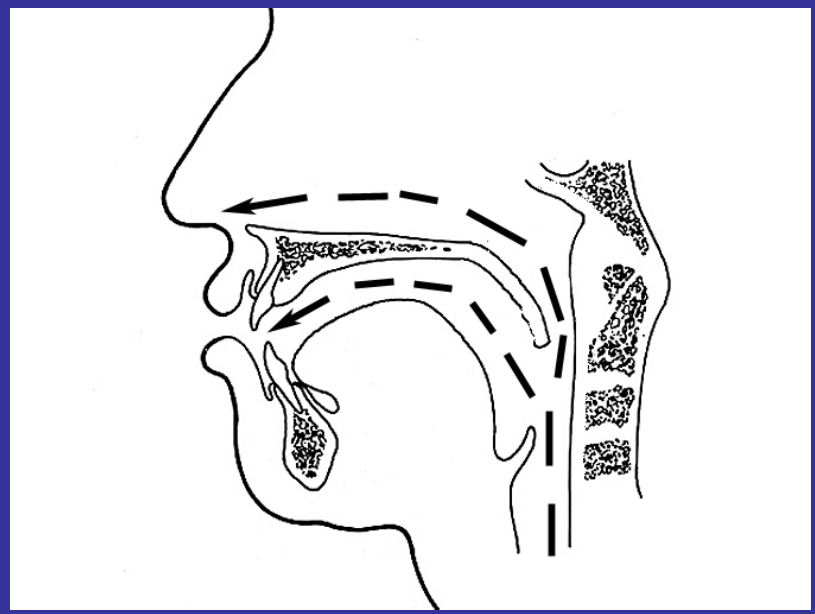
Cleft Palate Repair

- Day 85 of gestation
- Privileged period of scarless healing
- Secondary tissue layers repair also



Velopharyngeal Incompetence (VPI)





- VPI is characterized by absence of velopharyngeal closure.
- Velopharyngeal closure is accomplished by the levator veli palatini (LVP) muscle.

Hanes *et al.*, 2006

k_{tr} Determination of Fiber Type

Normal palate: 100% Type I (slow) fibers

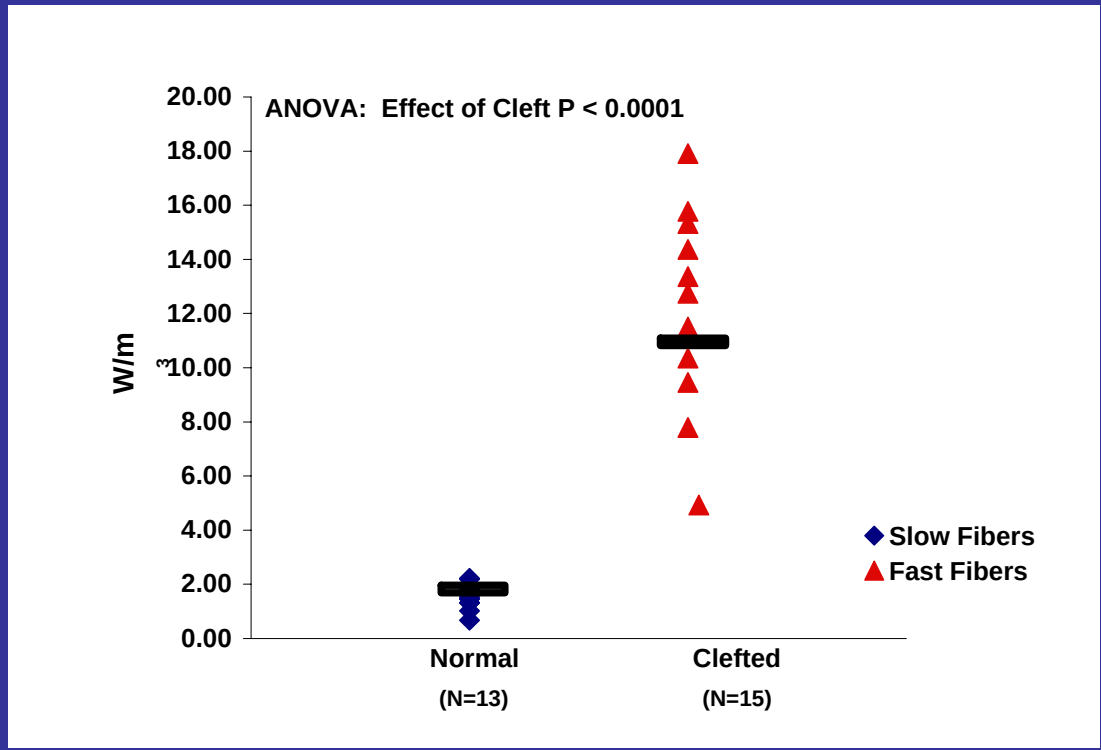
Cleft palate: 14% Type I (slow) fibers

86% Type II (fast) fibers

Numerous studies have shown that fast fibers differ significantly from slow fibers in their CSA, max. tetanic isometric force production, normalized power, and fatigability.

Hanes *et al.*, 2006

Maximum Normalized Power



Cleft palate muscle fibers produce 11x more normalized power than normal palate muscle fibers.

Hanes *et al.*, 2006

Conclusion

Fetal surgery is already approved when the life of the fetus is threatened.

As techniques are improved and the ethics and standards evaluated, I have no doubt that *in utero* fetal repair of the human cleft palate will become a reality.