

3 yrs Chihuahua female
accidentally mated



PREGNANCY DIAGNOSIS
22 days after mating

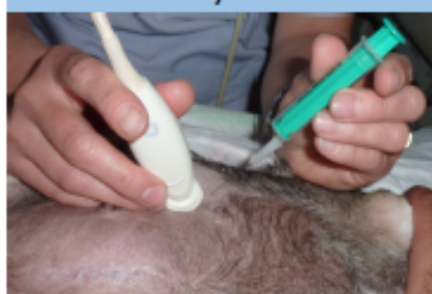


1. PREGNANT
2. CONFIRMATION OF PREGNANCY STAGE
3. ONLY ONE FOETUS

ULTRASOUND-GUIDED AMNIOTIC PUNCTURE

Female in dorsal recumbency – no tranquilization – no anesthesia
Ultrasound microconvex probe, 5-8 MHz, LOGIC 5 Expert, Scill Healthcare, General Electric Medical Systems
Clipping, disinfection of the area (chlorhexidin soap and solution)

Day 35



No post-operative sign

KARYOTYPING n1

(modified from Ducos et al, 1998)

Amniotic cell culture
in DMEM + 20% FCS + colcemid 2 h before harvesting.
Fixation (ethanol: acetic acid (3: 1))
Metaphase spreading (cold wet slides, air drying)
Giemsa staining



78 XX

Day 45



Normal D45 fetus
at ultrasound examination



No post-operative sign

KARYOTYPING n2

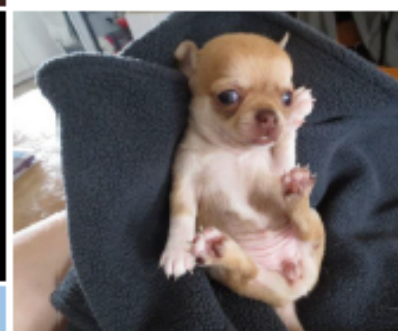
BLIND FOR THE CYTOGENETICS LAB
not aware the sample originated
from the same gestation/conceptus



78 XX

Day 65

SPONTANEOUS
DELIVERY
AT TERM



BIRTH
OF A NORMAL
FEMALE PUPPY

DISCUSSION

This paper shows the feasibility of **SEX DETERMINATION** thanks to **ULTRASOUND-GUIDED AMNIOCENTESIS** in the dog.

Ultrasound fetal sexing
is possible
but hardly feasible
Gil et al 2015, Prugnaud et al 2016

Intra-amniotic injections

of prostaglandin F2alpha
under ultrasound control
(Day35 pregnancy)
Manca et al 1996

of a retroviral gene vector
after laparotomy and
uterus exteriorization
(Day35 pregnancy)
Hayashita-Kihno et al 2015

WHAT WAS ACHIEVED PREVIOUSLY IN DOGS ?

Amniocentesis under visual control
during C section to evaluate fetal maturity
Maloni et al 2014, Bonte et al 2017, Bolla et al 2017, Veronni et al 2018

PERSPECTIVES

Besides fetal sex diagnosis, canine amniocentesis could also be used for prenatal genetic mutations identification.
Nevertheless, the main limit of the interests of this procedure in dogs is the presence of several fetuses per pregnancy
making it tricky to associate each prenatal diagnosis to the corresponding fetus or neonate.

Fetal sex determination by amniocentesis in the canine species: a case report

D.Layssol 1, A. Pinton 2, S. Chastant-Maillard 3

Ecole Nationale Vétérinaire de Toulouse, 23 Chemin des Capelles, 31076 TOULOUSE
CEDEX

1. Diagnostic Imaging ; 2. GenPhySE, Université de Toulouse, INRA, INPT, ENVT,
Castanet Tolosan, France; 3. NeoCare, UMR 1225 INRA/ENVT Interactions Hôte-Agent
Pathogène
s.chastant@envt.fr

Collection of fetal cells in suspension in the amniotic liquid by ultrasound guided aspiration (amniocentesis) is of routine use in humans for the determination of the fetal sex, identification of aneuploidy (e.g. trisomy 21) and identification of mutations. Such fetal diagnosis has never been performed in dogs. In the clinical case presented here, the cytological amniotic sample was analysed by caryotyping for fetal sex determination.

A 3 yrs Chihuahua female was accidentally mated by a male of the same breed. A pregnancy diagnosis was performed 22 days after mating, evidencing only one foetus, whose age was compatible with the indicated mating date. Thirty five days after mating, an amniocentesis was performed with the consent of the owner. The bitch was placed in dorsal recumbency in an adapted bed (Doggyrelax). The fetus and the amniotic membrane was clearly identified by transabdominal ultrasonography (microconvex probe, 5-8 MHz, LOGIC 5 Expert, Scill Healthcare, General Electric Medical Systems). A 23G sterile needle mounted on a 10 mL syringe was introduced under ultrasound control into the amniotic vesicle, at distance to any part of the fetus. Five milliliters of amniotic liquid were aspirated and immediately transferred to the lab for karyotyping. A second amniocentesis session was performed 15 days later with the same protocol and blind analyzed by the lab.

Cytogenetic analysis was carried out from culture of amniotic cells as previously described for fibroblasts [1]. Briefly, amniotic cells were cultured in DMEM medium, 20% fetal calf serum in 25 cm² dishes. Colcemid (final concentration 0.03 mg/l) was added to the culture 2 h before harvesting. Cells were collected using trypsin solution (0.25%), hypotonic treatment in new born calf serum: distilled water (1: 6) and fixation in ethanol: acetic acid (3: 1), chromosome preparations were spread on cold wet slides and air dried. Slides were stained with 3 % Giemsa solution in order to identify the sex chromosomes on the metaphases. The first cytological analysis concludes to a female fetus 78XX. The present bitch was observed during the hours following puncture and no clinical sign developed. Pregnancy continued normally during the following days. At the second ultrasound examination (15 days later), no clinical abnormality in the dam, nor any intra uterine growth retardation or fetal developmental or amniotic abnormality were noticed. The second cytological analysis, performed with the lab convinced that the sample was coming from another pregnant dam, also concluded to a female chromosomal formula. Sixty two days after mating, the bitch delivered normally a female fetus, of normal vitality and without any observed abnormality.

In the bitch, amniocentesis was described peroperatively during C section under visual control, to evaluate fetal maturity [2, 3]. But no paper reports the gestational use of amniocentesis in dogs for diagnostic purposes. The present paper demonstrates the feasibility in the canine species of a technique routinely performed in humans. Apart from fetal sex diagnosis, canine amniocentesis could also be used for prenatal genetic mutations identification. Nevertheless, the main limit of the interests of this procedure in dogs is the presence of several fetuses per pregnancy.

[1] Ducos et al, 1998, Hereditas 28: 221 -229; [2] Bonte et al 2017, Reprod Dom Anim.;52:1025–1029 [3] Veronesi et al, 2018, Theriogenology 108, 277e283



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