THE INCIDENCE OF INDIVIDUALS FREEMARTIN IN THE RIPOLLESA SHEEP BREED

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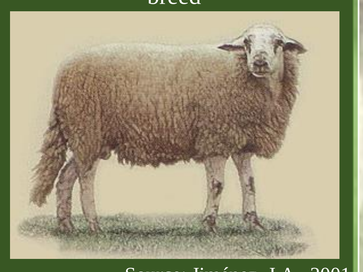
INTRODUCTION

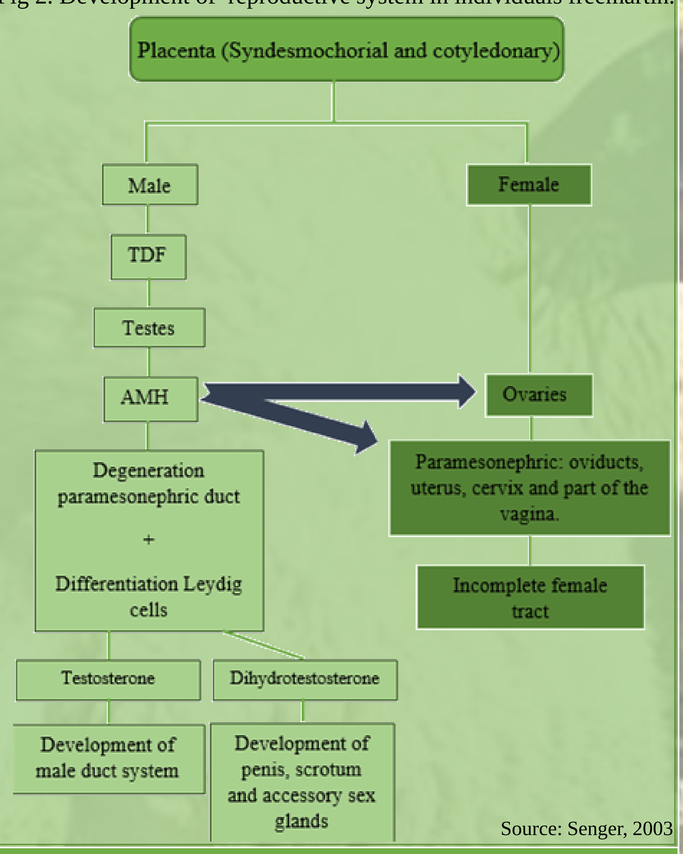
- The Ripollesa sheep breed is a Catalonia's autochthone breed that is in process of recuperation (2.35% of all Catalonian sheep census).
- There are measures to increase its census.
- Prolificacy: 1.1 – 1.6 lamp/gestation.
- The freemartinism is the most frequent form of intersexuality found, at ewes are associated to the discovery of high fecundity gens (more than 2 fetus).
- Incidence variable 1 – 3.58%.

Fig 1. Morphological characteristics of Ripollesa Sheep breed



Source: Jiménez, J.A., 2001

Fig 2. Development of reproductive system in individuals freemartin.



Source: Senger, 2003

OBJECTIVES

- Detection individuals freemartin, by the study of different techniques commonly used.
- Evaluation of new PCR real time technique for detection individuals freemartin.

DIAGNOSIS OF FREEMARTINISM

Different techniques for detection individuals freemartin

- Initially, ewes being born co-twin with a male sibling, history of failure to show estrous or be non pregnant after copulation.
- Clinical examination:
  - Clitoris enlarged.
  - Mammary glands and testes.
  - Vaginal length decreased.
  - Absence of definable cervix.
  - Abnormal size of gonads.

Fig 3. Clitoris enlarged

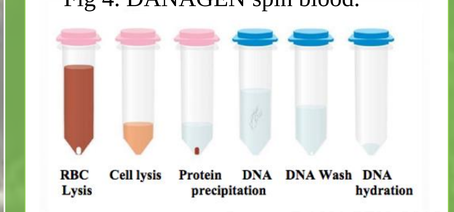
Source: Casellas, J. 2016

- Morphological and histological studies:
  - Macroscopy examination post-mortem.
  - Degeneration or fibrosis stromal tissue, no functionality.
- Karyotyping:
  - Lymphocyte culture for determine the sex chromosome chimeras XX/XY.
- Blood grouping (2 populations of RBC).
- Hormone methods (masculinization hypothalamus):
  - Not positive feedback LH response to estradiol treatment.
  - Abnormal FSH response to inhibin treatment.
- Detection of Y-Chromosome DNA:
  - PCR for amplification of DNA sequences marked for Y chromosome specific segments (DNA extraction previously and electrophoresis after).

MATERIALS AND METHODS

- 1140 blood samples from 1040 females and 100 males from 14 flocks.
- Jugular vein extraction using Vacutainer® tubes with EDTA and freeze the samples.
- DNA extraction:

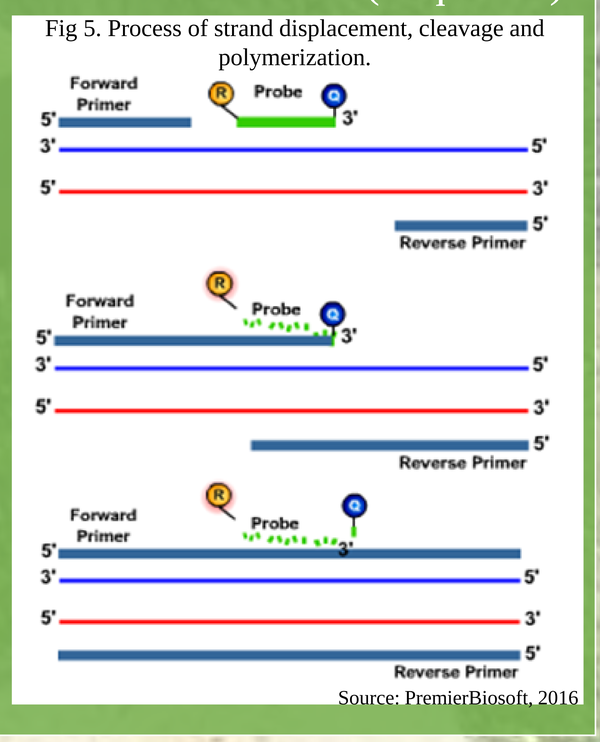
Fig 4. DANAGEN spin blood.



Source: DANAGEN, 2016

- PCR real time (TaqMan®):

Fig 5. Process of strand displacement, cleavage and polymerization.



Source: PremierBiosoft, 2016

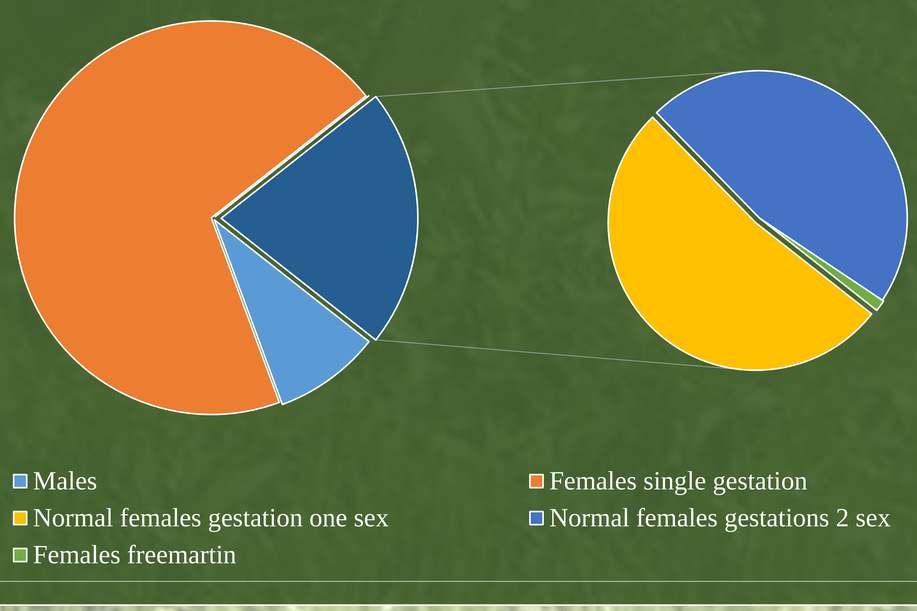
RESULTS

- 3 individuals freemartin.
- The incidence is variable because each flock has different census.
- The incidence real evaluate only ewes been born co-twin to a males.

Table 1.Incidence results obtained with the TaqMan technique.

Incidence	%
Flock ST	0.91
Flock JM	3.12
Flock XF	0.72
Total flocks	0.26
Real	2.58

Fig 6. Comparison of incidence results



DISCUSSION

- Incidence of freemartinism is low in the Ripollesa sheep breed because only observed twin gestations and the incidence in sheep increased with multiple gestation.
- The stockbreeders know better recognize the freemartin syndrome.
- PCRrt vs. other techniques.

CONCLUSIONS

- Incidence of individuals freemartin is similar than other studies in sheep, and this is low than cattle's incidence (90%).
- PCRrt is better than others techniques commonly used, more exactly and faster.