# Fertility rate evaluation by laparoscopic approach in the experimental animal

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## Summary

*Purpose of investigation:* The aim of the present study was to evaluate the effect of laparoscopic insemination (LAP) and natural mating (NM) on fertility rate in experimental animal *(Ovis Aries Comisana)* during the month of June.

*Methods:* For the experiment, 97 ewes were used. Laparoscopic insemination was performed with the frozen semen of three different Romanov rams: Laparoscopic insemination 1 (n = 24); Laparoscopic insemination 2 (n = 26); and laparoscopic insemination 3 (n = 28), and natural mating was performed with two different Ovis Aries Comisana rams with proven fertility: Natural mating 1 (n = 10); Natural mating 2 (n = 9). Estrus was synchronized with fluorogestone acetate impregnated intravaginal sponges (40 mg, 14 days). Pregnant mare serum gonadotrophin (Folligon, Intervet International) at a dose of 400 UI was given intramuscularly at sponge removal. Artificial insemination was carried out 60 hours after the removal of the sponges in the laparoscopic insemination groups.

*Results:* The mean pregnancy rate at ecographic diagnosis performed at about 36 days from sponge removal for the laparoscopic insemination and natural mating groups were respectively, 62.8% and 78.9% with no significant difference.

*Conclusion:* The mean fertility rates for the LAP and NM groups were 56.0 and 73.4, respectively, with no significant difference.

*Key words:* Fertility; Laparoscopic insemination; Experimental animal.

## Introduction

Artificial insemination (AI) of sheep with frozen semen is a basic need for genetic improvement through extensive exploitation of superior germplasm.

Unlike cattle, cervical insemination in sheep using frozen semen has not progressed due to low conception rates [1, 2, 3], whitch drastically reduce the impact of this technology on enetic improvement in Italy.

The major factor limiting fertility is the inability of frozen-thawed sperm to penetrate the complex cervix of the ewe [3, 4, 5]. On the contrary, the intrauterine laparoscopic technique of AI allows the direct deposition of semen in the uterus resulting in acceptable levels of fertility and lambing [6, 7, 8].

An important benefit of laparoscopy over transcervical insemination is that laparoscopy can be rapidly performed in all normal ewes. Therefore this technique was initially developed as a means of bypassing the cervix to facilitate the use of frozen-thawed semen when it was found that cervical insemination of frozen-thawed ram semen was associated with poor fertility.

The present trial was designed to evaluate the effect of AI performed with the frozen semen of three different rams and of natural mating performed with two different fertile rams on fertility and prolificacy in Gentile di Puglia ewes.

## Materials and Methods

The trial was carried out during the month of June at a Segezia farm near Foggia (41° lat. N. 74 m above sea level) characterised by a temperate winter climate (January, mean temperature, 8.7° C) and a dry-hot summer (July, mean temperature 24.3° C) with 400 mm annual rain fall; it involved 97 multiparous Gentile di Puglia ewes.

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The animals were assigned to five groups according to the frozen semen of three different rams (LAP1, LAP2, LAP3) for laparoscopic insemination and two different rams (NM1, NM2) for natural mating.

A constant diet (hay *ad libitum* and supplementary concentrate food) was given to the animals.

### *Synchronization*

Estrus synchronization, achieved using 40 mg FGA sponges (Crhonogest, Intervet, Italy), was administered by intramuscular injection at the time of sponge removal. Laparoscopic insemination was performed 60 h after completion of sponge removal; the ewes of the natural mating groups were placed with two rams with a good history of fertility 20 h after sponge removal.

### *Semen*

The frozen semen used for laparoscopic insemination was provided by Société Coopérative Agricole, Montmorillo, INRA (Institut National de Recherche Agronomique), France, and was commercially available to the sheep industry.

The frozen semen was supplied in 0.25 ml PVC (Polyvinyl chloride) straws; the post-thaw traits of semen used for insemination are reported in Table 1.

### *Artificial insemination*

The ewes were fasted overnight prior to laparoscopic insemination.

Ten minutes before laparoscopy, local anaesthesia (5 ml of 2% lidocaine, Fort Dodge, Italy) was provided at the trocar insertion.

Table 1. — *Qualitative analysis of frozen-thawed ram semen used for insemination in ewes.*

| Ram semen | TSE a | MOT b (%) | PMOT c (%) | MPV d | MSE e |
|---|---|---|---|---|---|
| LAP1 | 96.5 | 56.0 | 46.0 | 107.0 | 44.4 |
| LAP2 | 119.9 | 51.0 | 39.0 | 95.0 | 46.8 |
| LAP3 | 83.5 | 66.0 | 55.0 | 94.0 | 45.9 |

a TSE: Total number of spermatozoa inseminated per ewe (x $10^6$)
b MOT%: Percentage of motile spermatozoa inseminated per ewe
c PMOT%: Percentage of progressively motile spermatozoa
d MPV: Mean progressive velocity of spermatozoa (microns per second)
e MSE: Number of motile spermatozoa inseminated per ewe (x $10^6$)

The ewes were restrained in the dorsal recumbent position in a laparotomy cradle and held at a 45° angle with the head downward; the abdomen was insufflated with a $CO_2$ cylinder with an attached flow meter. A 7 mm laparoscope (30°) was inserted into a cannula through another stab incision. Once the uterus was visualized the needle was placed in the uterine lumen of the mid-section of the greater curvature of one horn, the inselinate expelled and the needle withdrawn. The procedure was then repeated in the other horn: each dose was divided equally between the two uterine horns. No attempt was made to inspect the ovaries to determine the side of ovulation.

Excess $CO_2$ was expressed from the abdomen after withdrawal of the laparoscopic equipment, and the ewe was allowed to return to covered yards.

*Pregnancy diagnosis*

Pregnancy diagnosis was performed by transvaginal ultrasound 36 days after insemination and by observation of lambing 147-155 days after insemination [9].

For the examinations the ewes were restrained briefly in a brace designed for sheep handling with a 5 MHz probe. It was not necessary to use sedatives to immobilize the animals.

Ewes were diagnosed as either pregnant or not pregnant and an attempt was made to determine fetal numbers.

*Statistical analysis*

Data were recorded on inseminations and pregnant ewes for each ram using the following parameters: proportion of pregnant ewes out of inseminated ewes, number of fetuses out of pregnant ewes, and lambing ewes out of inseminated ewes. Analysis by the chi-square test corrected by the Yates test was carried out [10].

## Results

Lambs produced from the laparoscopic AI were born over a 4 to 7-day period; those produced from natural mating were born over an 8-day period.

The average rate of pregnant ewes determined by ultrasound was 62.8% for LAP groups and 78.9% for NM groups without significant differences.

No difference was seen in pregnancy rates between rams, with a minimun of 57.7% in the LAP2 group and a maximum of 90% in the NM group.

The proportions of single and twin fetuses at ultrasound were 57.1% vs 42.9% for the LAP and 53.3% vs 46.7% for the NM group without significant differences (Table 2).

The fertility rate at lambing for the LAP and NM groups were 56.1% and 73.4%, respectively, without significant differences.

The fecundity and prolificacy rates for the LAP and NM groups were 73.9% vs 89.5% and 116.3% vs 113.3%, respectively. The insemination technique of the rams did not show significant differences.

Out of 49 LAP pregnant ewes diagnosed at ultrasound, 44 animals delivered (89.9%) and out of 15 NM ewes, 15 animals delivered (93.3%).

The proportions of single and twin lambs at lambing were 63.3% vs 26.5% for the LAP group and 73.3% vs 20.0% for the NM group without significant differences (Table 3).

## Discussion

This is the first report on laparoscopic artificial insemination in experimental animals (Gentile di Puglia ewes). In this breed the laparoscopic technique could be interesting because a pregnancy rate not significantly different from the one obtained by natural mating was achieved. The resulting fertility rates of AI in the synchronized estrus are similar to those reported by other authors [11, 12] but are slightly lower than those reported in other experiments [8, 13].

AI results may be influenced by various factors such as estrus response, semen used and stress.

Estrus detection was not performed in this study, therefore the number of ewes in estrus at the time of inse-

Table 2. — *Ultrasound results following insemination in ewes using either a laparoscopic (LAP) or the natural mating (NM) technique.*

| Technique | No. Inseminated | Ultrasound Diagnosis (a) | | | | Fetuses (b) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Pregnant | | Not Pregnant | | Single | | Twin | |
| | | no. | % | no. | % | no. | % | no. | % |
| Laparoscopic | | | | | | | | | |
| LAP 1 | 24 | 16 | 66.7 | 8 | 33.3 | 9 | 56.3 | 7 | 43.7 |
| LAP 2 | 26 | 15 | 57.7 | 11 | 42.3 | 9 | 60.0 | 6 | 40.0 |
| LAP 3 | 28 | 18 | 64.3 | 10 | 35.7 | 10 | 55.6 | 8 | 44.4 |
| Total | 78 | 49 | 62.8 | 29 | 37.2 | 28 | 57.1 | 21 | 42.9 |
| Natural Mating | | | | | | | | | |
| NM 1 | 10 | 9 | 90.0 | 1 | 10.0 | 2 | 22.2 | 7 | 77.8 |
| NM 2 | 9 | 6 | 67.7 | 3 | 33.3 | 6 | 100.0 | 0 | 0 |
| Total | 19 | 15 | 78.9 | 4 | 21.0 | 8 | 53.3 | 7 | 46.7 |

(a): No. of pregnant ewes or not inseminated ewes. (b): No. of fetuses/pregnant ewes.

Table 3. — *Lambing results following insemination using either laparoscopy (LAP) or the natural mating (NM) technique.*

| Technique | Examination at lambing (a) | | | | Lambs (b) | | | | Fecundity (c) | Prolificity (d) |
|---|---|---|---|---|---|---|---|---|---|---|
| | Lambing | | Not lambing | | Single | | Twin | | | |
| | no. | % | no. | % | no. | % | no. | % | % | % |
| Laparoscopic | | | | | | | | | | |
| LAP 1 | 12 | 50.0 | 4 | 16.7 | 10 | 62.5 | 2 | 12.5 | 58.3 | 87.5 |
| LAP 2 | 15 | 57.7 | 0 | 0 | 10 | 66.7 | 5 | 33.3 | 76.9 | 133.3 |
| LAP 3 | 17 | 60.7 | 1 | 0.03 | 11 | 61.1 | 6 | 33.3 | 82.1 | 127.3 |
| Total | 44 | 56.1 | 5 | 5.06 | 31 | 63.3 | 13 | 26.5 | 73.1 | 116.3 |
| Natural Mating | | | | | | | | | | |
| NM 1 | 8 | 80.0 | 1 | 0.01 | 6 | 66.7 | 2 | 22.2 | 100.0 | 111.1 |
| NM 2 | 6 | 66.7 | 0 | 0 | 5 | 83.3 | 1 | 16.7 | 77.8 | 116.7 |
| Total | 14 | 73.4 | 1 | 0.05 | 11 | 73.3 | 3 | 20.0 | 89.5 | 113.3 |

(a): Lambing ewes/inseminated ewes. (b): No. of lambs/pregnant ewes at ultrasound. (c): No. of lambs/inseminated ewes. (d): No. of lambs/lambing ewes.

mination was unknown. Thus there is a possibility that some ewes were not in estrus or that the time of insemination was not appropriate for some ewes.

However, the semen used in all insemination groups was adeguate in motility and concentration of spermatozoa.

The stress factor involved in handing the ewes at the time of insemination could also affect the fertility obtained because the animals were not accustomed to being regularly handled.

From this study we noted a low variation in the pregnancy rate of ewes inseminated with the semen from different rams; in fact we found a range in fertility between rams of 10.7% (50 to 60.7%).

Previous studies found a range in fertility between rams of 28% (26 to 54%), 26% (51 to 77%) and 69% (17 to 86%) using 3, 14 and 110 rams, respectively [14, 15].

Moreover, because fertility was determined early (36 days after insemination) we noted that the percentage of ewes with cases of embryo mortality did not differ significantly between the two techniques (16.4 vs 26.7% for LAP and NM groups, respectively).

Comparison between twin fetuses observed by ultrasound and by lambing were not different for the LAP and NM techniques (16.4 vs 26.7% for the LAP and NM groups, respectively).

## Conclusion

The results of the present experiment suggest that the reproductive performances of ewes inseminated by either the laparoscopic or natural mating technique were not affected by either insemination technique or by individual rams within the technique.

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