
*Unité de Physiologie de la Reprod. et des Comportements, INRA, 37380 Nouzilly, France

Abstract

Seven bucks from Serrana breed were used for semen collection with artificial vagina along the year. Two hundred and eighteen ejaculates of good quality were collected, washed to remove seminal plasma, refrigerated (4 ºC) and frozen in nitrogen vapours. Seminal traits were evaluated in fresh and frozen semen. Cervical insemination with frozen semen (n=35) or refrigerated semen (n=251) was done 43-45 hours after sponge removal and fertility and fecundity were evaluated. Frozen semen of two bucks x two seasons was tested on in vitro fertilization. In fresh semen individual differences were detected for volume, concentration, live, normal and sperm abnormalities and seasonal variation was found for volume, normal and mid piece abnormalities. Freezing decreased significantly all sperm traits except for mid piece abnormalities. In frozen/thawed semen, individual variations were detected in almost all seminal traits with exception of mid piece sperm abnormalities and seasonal variations were observed for sperm abnormalities, and for Host values at 5 and 25 minutes. In goats inseminated with refrigerated semen, fertility, fecundity and prolificacy were respectively 60.2 %, 106.0 % and 176.1 % with differences among males. Positive correlations between individual motility, live and normal sperm with fertility were found. Reproductive parameters in goats inseminated with frozen semen were respectively 27 %, 56 % and 208 %. At 24 hours post in vitro insemination (pi), independently of season of freezing, one of the bucks showed higher cleavage rates (39.9% vs. 25.3%, respectively, P<0.05) and produced significantly higher embryo rates at D6, D7 and D8 (P< 0.05). At 48 hours post-fertilisation, these differences among animals were no longer significant. Cleavage at 24 h (48.0% vs. 22.9%) and D6 (58.1% vs. 14.7%) rates of autumn frozen semen were also higher in the former best buck (P<0.05). Higher cleavage rate was observed in autumn than in winter frozen semen in one buck. At D7 and D8, seasonal effect on embryo rates was not significant.

Keywords:  Serrana breed, frozen semen, seminal traits, AI, in vitro fertilization

Introduction

The Serrana goat breed is located at the north and centre of Portugal under semi extensive conditions and is exploited for milk and cheese production. Ovarian inactivity exists between January and the end of May, with a deeper anoestrus in February and March (Mascarenhas et al., 1992). There are two breeding periods, one in May-June and the other in August-October. In goats delivering in march/april, seasonal anoestrus is confounded with lactational anoestrus till may/june. Puberty either in males and females is influenced by seasonal conditions (Horta et al., 1987, Baptista et al., 1991). Fertility rates obtained with natural service are 89.3 % (Azevedo et al., 1993) and with artificial insemination using refrigerated semen varies from 47 to 63 % with most values around 58-60 % (Azevedo et al., 1993,
Mascarenhas & Barbas, 2000). No reports of artificial insemination (AI) with frozen semen are available in Serrana breed. There are individual variations in most seminal traits in fresh semen and some of them are influenced by season with higher values for live and normal sperm during the decreasing photoperiod (Sousa et al., 2001). In vitro fertilization has been performed in bulls and rams in order to investigate its potential as predictor of the in vivo fertility with frozen-thawed semen (Papadopoulos et al., 2005). No reports are available of in vitro fertilisation tests carried out for this purpose with buck semen from Serrana breed. The objectives of this work were the characterization of seminal traits from fresh and frozen semen around the year, the fertility rates after AI using refrigerated semen and frozen semen and ability of semen frozen in autumn and winter to fertilize oocytes matured in vitro, in the Serrana breed.

**Material and Methods**

Seven bucks from Serrana breed, were used for semen collection with artificial vagina around the year during two years. Seminal traits of fresh and frozen/thawed semen were evaluated according to Evans & Maxwell (1990). Two hundred and eighteen ejaculates of good quality based on individual motility (IM>65%) were collected. After evaluation, kreb’s-ringer-phosphate-glucose solution was used to wash semen according to Leboeuf (2001). Fresh semen was diluted and packed in 0.25 mL Cassou straws (200 x 10⁶ spz/straw) and refrigeration lasted 4 h. Refrigerated semen (+4 ºC) was frozen in a cryo-chamber before LN₂ immersion. Host test (Hypoosmotic swelling test) was performed in thawed semen (5, 25 and 40 minutes) according to Artiga (1994). If thawed semen was in good conditions namely ≥40 % IM and < 20 % sperm abnormalities it could be used for artificial insemination (AI). In this work fresh ejaculates were prepared to make AI with refrigerated semen (15 ºC) according to Chemineau et al. (1993). Oestrus synchronization was done with fluorogestone acetate (45 mg) vaginal sponges during 11 days according to Leboeuf (2001). AI with refrigerated semen (15 ºC) or with frozen semen was done respectively in 251 or 45 Serrana goats. Cervical AI with frozen semen (2 bucks) was performed 43-45 h after sponge removal using 2 straws or with refrigerated semen (8 bucks) using one straw per goat. Fertility, fecundity and prolificacy were evaluated in the inseminated goats.

Abbatoir goat ovaries in Ribatejo region are not abundant. Consequently, in vitro fertilisation of in vitro matured oocytes with autumn (n=363 oocytes) and winter (n=373) frozen semen from two Serrana bucks was performed at INRA, Nouzilly-France. Aspirated oocytes from adult French goat ovaries were matured in TCM199 plus 100µM cysteamine and 10 ng mL⁻¹ epidermal growth factor (EGF) in a 5% CO₂ incubator at 39°C for 22 h (Cognié et al., 2004). Motile thawed spermatozoa were obtained by centrifugation on a Percoll density gradient. Sperm was resuspended (10⁷ spz mL⁻¹) with synthetic oviductal fluid (SOF) plus 10% estrus sheep serum (ESS) and 4 µl mL⁻¹gentamicine and incubated for 30 min at 39°C in 5% CO₂. Capacitated spermatozoa (10⁶ spz mL⁻¹) were co-incubated with matured oocytes in SOF plus 10% ESS for 18 h at the same conditions. After fertilisation, zygotes were transferred to SOF droplets with 10% bovine serum albumin in an atmosphere of 5% CO₂, 90%N₂ and 5%O₂ at 39°C. At 48 h post insemination (pi), SOF was supplemented with 10% ESS. Cleavage at 24 and 48 h pi (cleaved embryos /inseminated oocytes), embryos at day 6 (D 6), D7 and D8 of culture (embryos /cleaved embryos) and hatched embryo rates (hatched embryos/ D7 embryos) were defined.
Results

Average and 95% confidence limits (CL) of fresh and thawed seminal parameters computed independently of seasonal and individual factors (ejaculates from 7 bucks) are presented in table 1. Freezing decreased significantly all semen traits with exception of tail piece abnormalities. All the homologous traits between fresh and frozen semen were significantly correlated, particularly individual motility (IM), tail and mid piece abnormalities.

Table 1. Overall results for fresh and frozen/thawed semen traits.

<table>
<thead>
<tr>
<th></th>
<th>Fresh semen</th>
<th>Thawed semen</th>
<th>Difference (thawed – fresh)</th>
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<tr>
<td></td>
<td>N* Mean 95% CL</td>
<td>N Mean 95% CL</td>
<td></td>
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<tr>
<td>Volume (mL)</td>
<td>218 1.09 1.04-1.15</td>
<td>214 3.85-4.23</td>
<td></td>
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<tr>
<td>Conc. (x 10^9 mL^-1)</td>
<td>218 4.04 3.85-4.23</td>
<td>218 4.45 43-46</td>
<td>-23.1</td>
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<tr>
<td>Ind Motility (%)</td>
<td>218 65.1 64.4-65.8</td>
<td>218 64.9 63.8-66</td>
<td>-27.7</td>
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<td>Live spz (%)</td>
<td>216 72.2 70.8-73.6</td>
<td>218 62.8 57.8-66</td>
<td>-16.2</td>
</tr>
<tr>
<td>Normal spz (%)</td>
<td>216 81.1 80.1-82.1</td>
<td>218 64.9 63.8-66</td>
<td>-16.2</td>
</tr>
<tr>
<td>Head Abn. (%)</td>
<td>216 9.3 8.6-9.9</td>
<td>218 26.8 25.7-27.9</td>
<td>+20.8</td>
</tr>
<tr>
<td>IP Abn (%)</td>
<td>216 5.2 4.6-5.7</td>
<td>218 2.4 2.0-2.8</td>
<td>-2.8</td>
</tr>
<tr>
<td>Tail Abn (%)</td>
<td>216 4.4 3.9</td>
<td>218 5.8 5.1-6.4</td>
<td>+1.9</td>
</tr>
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</table>

1 Data lost from 2 fresh ejaculates but included in thawed semen
2 Traits differing significantly between fresh and frozen semen (ANOVA; P<0.05)

Seasonal variations in fresh semen were seen for volume, normal sperm and mid piece abnormalities with better performances in the autumn. Individual variations (p<0.03) were observed for volume, concentration, live, normal, and all sperm abnormalities. In thawed semen all seminal traits with exception of mid piece sperm abnormalities were different among animals (p<0.006). In frozen/thawed semen, seasonal variations were observed for all sperm abnormalities, Host 5 and Host 25 values. In the autumn there were higher sperm head abnormalities and during spring and winter more mid piece and tail sperm abnormalities. Higher values of Host 5 and Host 25 were observed in winter and spring. The difference of values between fresh and thawed semen for normal sperm were significantly higher in the autumn than in summer, winter or spring (18.9 % vs. 14.5 % or 14.3 or 11.5 %, respectively; p<0.001). Interactions of season with semen type (fresh or frozen) were found for normal sperm (p<0.01), head (p<0.002) and mid piece sperm abnormalities (p<0.05) with different seasonal variations between fresh and frozen semen particularly for normal sperm and head sperm abnormalities.

In fresh semen the interaction of seasons (autumn, winter, spring and summer) with bucks (n=3) were significant for IM, live sperm and tail sperm abnormalities (p<0.03). In frozen semen the interaction of seasons with bucks were significant for IM, live and normal sperm, mid piece abnormalities and Host 25-40 values. Fertility, fecundity and prolificacy after artificial insemination performed during spring (n=251) with refrigerated semen (15 ºC) from eight Serrana bucks were significantly different among bucks. Globally these reproductive parameters were 60.2 %, 106.0 % and 176.1 %, respectively. Higher fertility and fecundity rates were achieved in three bucks. In three bucks used in AI we did not see individual variations (fresh semen) concerning IM, live and normal sperm. Positive correlations between IM (r=0.82), live (r=0.999) and normal sperm (r=0.795) with fertility
were determined, with significant correlations between live sperm and fertility. Forty five goats were inseminated with frozen thawed semen from two bucks. Fertility, fecundity and prolificacy were respectively 27%, 56% and 208%, without differences among bucks.

At 24 hours post in vitro insemination (pi), independently of the freezing season, buck 711 showed higher cleavage rates than buck 131 (39.89%±11.68% vs. 25.26%±7.12%, respectively, P<0.05). The former buck achieved higher cleavage rates at the same stage when its semen was frozen in autumn than in winter (48.0%±10.98% vs. 31.78%±4.70%, respectively, P<0.05). In autumn, frozen semen from buck 711 was also superior to buck 131 (cleavage rates: 48.0%±10.98% vs. 22.91%±8.01%, respectively, P<0.05). At 48 hours post-fertilisation, these differences among animals and seasons were no longer significant. Buck 711 also produced significantly higher embryo rates at D6, D7 and D8 than buck 131 (P<0.05), regardless the season of semen cryopreservation. No differences between the two bucks were found for hatched embryo rates. In vitro fertilisation with autumn semen from buck 711 produced significantly higher number of embryos than autumn semen from buck 131 (58.08%±11.42% vs. 14.70%±3.81%, respectively, P<0.05). At D7 and D8, seasonal effect on embryo rates was not significant.

Discussion

In fresh semen all seminal traits were different among animals with exception of IM which also varied in other studies (Sousa et al., 2001). Seasonal variations were found for volume, normal sperm and mid piece abnormalities with higher performances in autumn followed by summer in agreement with Sousa et al. (2001). Positive correlations between live and normal sperm were found in this and other studies (Ferreira, 2000). There are no reports in this breed about seminal traits evaluation in frozen/thawed semen. Individual variations were observed in frozen semen for all seminal traits. All sperm abnormalities, Host 5 and Host 25 values showed seasonal variations. Tail and mid piece abnormalities in frozen semen were higher in spring and winter while head abnormalities were higher in autumn. Higher values of Host 5 and 25 were detected in winter and spring but with fresh semen no seasonal variations were found (Ferreira, 2000). The difference between fresh and frozen semen found in the autumn for normal sperm was due to higher values found in fresh semen and does not reflect a depressive effect on frozen semen. Except for tail abnormalities, all seminal traits were worse in frozen semen than in fresh semen which is in accordance with other authors (Donavan et al., 2004). Interactions of season with semen type (fresh or frozen) were found for normal sperm, head and mid piece abnormalities. In winter fresh semen there was an increase in mid piece abnormalities and a decrease in normal sperm according with others (Azerêdo et al., 2001, Gillan et al., 2004). Results on fertility of inseminated goats with refrigerated semen are in accordance with previous reports (Azevedo et al., 1993, Mascarenhas & Barbas, 2000) showing individual variation between bucks. Results obtained in Serrana goats with frozen semen are encouraging and did not show individual variations between bucks. Results of artificial insemination with refrigerated semen were higher than with frozen/thawed semen and are in accordance with others (Gillan et al., 2004).

Differences found on cleavage rates between the two bucks at 24 hours post in vitro insemination, although not present at 48 hours, persisted further in culture through the number of embryos produced at D6, D7 and D8. These differences are consistent with those showed by Morris et al. (2003) in rams, suggesting that other intrinsic sperm factors besides motility are not excluded by the Percoll gradient or swim up techniques. With autumn frozen semen, differences between males were only evident on cleavage rates at 24 h pi and embryo rates at D6 of culture, suggesting that individual rather than seasonal factors influence in vitro embryo production.
References


