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Fertility prediction in dairy goats from Murciano-Granadina breed: The role of sperm evaluation and female traits

E. Mocé [a,d], M.L. Mocé [b,d], S.A. Lozano-Palazón [c], J. Bernácer [a,d], M.M. Martínez-Granell [a,d], I.C. Esteve [a,d], F. Bernat [a,d], S.J. Contreras [a,d], I. Villalba [a,d], E.A. Gómez [a,d,*]

[a] CITA-Instituto Valenciano de Investigaciones Agrarias, Polígono la Esperanza, 100, 12400 Segorbe, Castellón, Spain
[b] Department of Animal Production and Health, Veterinary Public Health and Food Science and Technology (PASAPTA), Facultad de Veterinaria, Universidad Cardenal Herrera-CEU, CEU Universities, C. Tirant lo Blanc, 7, 46113, Alfara del Patriarca, Valencia, Spain
[c] ACRIMUR, C. Barón del Solar, 22-A, Edificio II, Entresuelo A, Puerta B, 30520 Jumilla, Murcia, Spain
[d] Unidad Asociada UCH-CEU – IVIA, Valencia, Spain

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ABSTRACT

Fertility is one of the most economically important traits in farm animals, due to the direct and indirect costs associated to low pregnancy rates. Thus, one of the priority goals in animal reproduction is to predict the performance that the semen doses will have *in vivo* based on the quality values obtained in laboratory assays. Attempts have been made for getting a predictive model of fertility of frozen-thawed sperm in dairy goats, but similar studies have not been conducted for chilled goat buck sperm doses that are mostly used for artificial insemination in many countries including Spain. We study how parameters of *in vitro* sperm quality and characteristics of Murciano-Granadina dairy goats may affect the *in vivo* fertility obtained after artificial insemination with semen doses chilled at 4 °C. Moreover, this information was used for obtaining predictive models of the fertility. Sixty-three ejaculates from 13 males were used to prepare chilled doses for the insemination of 495 goats over 13 sessions. Fresh and chilled sperm were evaluated for motility and plasma membrane integrity with a computer-assisted sperm analysis system and flow cytometry, respectively. Fertility was determined at parturition, according to the kidding goats. Overall fertility was 59.6%. Pearson's correlation coefficients between *in vivo* fertility and quality variables of fresh sperm were not significant and were low (below 0.34 in absolute value) for chilled sperm. Females' characteristics had a low negative impact on fertility (correlation coefficients of −0.19 with age, −0.20 with parturitions and −0.11 with total milk yield obtained in the best lactation). Fixed and mixed logistic regression procedures were used trying to explain the fertility results. None of the models accurately predicted fertility, but the best models included the percentage of total motile sperm or average path velocity from fresh semen, age of the females and the session effect (uncontrolled environmental effects). These analyses showed that primiparous goats were 2.42 times more likely to get pregnant than goats that had kidded four or more times. Our field assay data on fertility in Murciano-Granadina dairy goats highlighted the importance of making quality controls of sperm, of choosing the doses presenting high percentages of motile sperm exhibiting regular trajectories and of selecting the youngest goats for AI, after their first kidding. Efforts should continue to obtain better predictive models for improving fertility in goat dairy herds.

Implications

Fertility of inseminated goats is a critical parameter. It correlates negatively with the age of females and positively with the percentage of total motile sperm and the average path velocity. Insemination results are affected by uncontrolled external factors and models for predicting fertility are far from being optimum. In base to these results, we recommended that the youngest females (after first kidding) should be selected for insemination and the doses delivered should be chosen based on the presence of a high percentage of motile sperm exhibiting regular trajectories. Furthermore, the identification of extrinsic factors that may affect fertility is mandatory.

* Corresponding author.
E-mail address: gomez_ern@gva.es (E.A. Gómez).

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Introduction

Fertility is one of the most economically important traits in livestock (Taylor et al., 2018). Low pregnancy rates provoke direct economic losses associated to the cost associated to the artificial insemination (**AI**; i.e. hormones for oestrous synchronisation, semen doses and the technician visit). Moreover, delayed conceptions cause non-immediate economic losses (Barth, 2018; Kumaresan et al., 2020) because dairy goats that conceive late will have more unproductive days due to delayed lactations and the culling cost of animals will also increase (in goats: Belanche et al., 2019; in cows: Fetrow et al., 2006; Hadley et al., 2006) penalising the herd-life economic efficiency (Malher et al., 2001). Therefore, recognising the factors that can determine fertility is economically very important.

One of the measurable factors that affect the fertility is the quality of the sperm doses used in AI (Broekhuijse et al., 2012a). Among the parameters that affect the quality of ejaculates, motility is possibly the quality parameter more widely used. Sperm needs to be motile for its transport through the female genital tract and for penetrating the oocyte vestments (Graham and Mocé, 2005). Indeed, samples with lower motility will have lower or limited capacity to fertilise oocytes (Yániz et al., 2018). Computer-aided motility analysers provide objective evaluation of the quantity of the sperm that are motile, as well as of the quality of that movement (providing velocities and indexes; Yániz et al., 2018). Furthermore, the sperm plasma membrane plays important roles in capacitation and signalising events. An intact and competent plasma membrane for fertilising oocytes is necessary (Graham and Mocé, 2005; in sheep: Mendoza et al., 2021). This structure is objectively and rapidly evaluated by means of fluorescent stains and flow cytometry (Graham and Mocé, 2005).

Obviously, animal fertility also depends on females. In previous research on cattle, the selection for milking yield has decreased their fertility (Royal et al., 2000; Rajala-Schultz and Frazer, 2003; Berry et al., 2014; Ma et al., 2019) thus provoking reproductive problems in high-producing lactating dairy cows (Lucy, 2001; Lonergan et al., 2016). In general, the genetic correlation between milk production and reproductive performance is antagonistic (Berry et al., 2014). Previous research in dairy goats reported that selection per milk yield in Alpine breed brought about a decrease in fertility after AI, although this trend was not observed in Saanen breed (Furstoss et al., 2015). Moreover, recent reports in Murciano-Granadina breed indicated that fertility was affected in goats yielding more than 3 250 mL milk/day at mating (Fernández et al., 2021).

In dairy goats, the oestrous is synchronised and AI is performed at a fixed time after pessaries are removed, therefore, the AI is standardised and the fertility data are accurate. Moreover, the protocols for preparing and analysing the sperm quality are standardised and the males are used to inseminate females located in different herds, so the animals are representative of the population, which are some of the critical points for analysing field fertility (Broekhuijse et al., 2012a).

One of the priority goals in animal reproduction is to predict the performance that the semen doses will have *in vivo* based on the quality values obtained in laboratory assays. Attempts have been made in different species for getting a predictive model of the fertility of fresh-refrigerated or frozen-thawed semen (in rabbits: Lavara et al., 2005; in pigs: Broekhuijse et al., 2012b; in rams: Santolaria et al., 2015; in bulls: Nagy et al., 2015; among others), although all of them failed to fully explain the fertility obtained. To the best of our knowledge, there is only one study in goats for the validation of a simple test to predict the fertility of frozen-thawed doses (Furstoss et al., 2010), but similar studies have not been conducted for chilled doses that are mostly used for AI in this species, at least in Spain (Mocé et al., 2020).

The general objective of this work was to study how parameters of *in vitro* sperm quality and characteristics of dairy goats may affect the *in vivo* fertility obtained after artificial insemination with semen doses chilled at 4 °C fitting predictive models for the fertility. Then, this information would be useful to improve fertility optimising, as possible, the most influential sperm parameters or goat characteristics.

Material and methods

Animals

Feed, housing and breeding conditions for males and females were similar to those described in Mocé et al. (2020). Sperm doses were provided by 13 adult Murciano-Granadina goat males housed in the Centro de Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias (Segorbe, Castellón, Spain). Animals participated in the national breeding programme of this breed. Animals were housed in pens and were fed straw and lucerne and a daily complement of 1 kg concentrated feed (CP 17%, crude oils and fat 4.5% and crude fibre 11.6%) per male. Fresh water was provided *ad libitum*. Animal housing, care and protocols for semen collection were approved by the Animal Care and Use Committee of Centro de Tecnología Animal, Instituto Valenciano de Investigaciones Agrarias and fulfilled the European regulations for the care and use of animals for scientific purposes (Real Decreto 53/2013, BOE, 2013). The experiment was conducted from February until December 2017.

Adult (>18 months) multiparous (≥1 parturition) Murciano-Granadina goats (n = 495) belonging to the breeder association Asociación Española de Criadores de la Cabra Murciano Granadina were used for the fertility trial. They were separated into 12 herds in different locations at distances ranging from 87 km (50 min) to 384 km (3 h 47 min) from the AI centre. Females were bred under an intensive production system for dairy-oriented herds that were housed in collective yards and managed according to their milk yield and reproductive state. They were provided concentrated feed (adapted to the production level), straw and lucerne. Fresh water was provided *ad libitum*.

Females' information was provided by Asociación Española de Criadores de la Cabra Murciano Granadina: age (age at the moment of AI measured in days), characteristics of milk yield (Max milk (kg) as total milk yield obtained in the best lactation from the female; Milk yield per lactation (kg) as total milk (kg) produced in all the lactations/number of lactations; Fat-protein (kg) as total kg of fat and protein produced in all lactations/number of lactations), parturitions (number of parturitions between 1 and 11 were categorised into four levels: 1, 2, 3 and > 3 parturitions, with 163, 134, 87 and 111 goat does in each level), breeding values (**GV**) were estimated in the official breeding programme (GV milk, as breeding value for milk yield/lactation, in kg; GV fat, as breeding value for fat production/lactation, in kg; GV protein, as breeding value for protein production/lactation, in kg; and the index combining milk yield and composition (**ICOt**)) and type of sperm (AI doses chilled to 4 °C during transport (using a portable compressor cooler) or in the laboratory (in a programmable water bath)).

Semen collection, preparation of chilled artificial insemination doses and in vitro sperm quality evaluation

Ejaculates were collected using an artificial vagina, as described in Silvestre et al. (2004), early in the morning. The chilled doses were prepared with a skimmed milk diluent in accordance to the

protocols described in Mocé et al. (2020). Briefly, the concentration in each ejaculate was adjusted to 560 × 10^6 sperm/mL with skimmed milk diluent (~22 °C), keeping an aliquot of 40 µL from each diluted ejaculate to perform the analyses of the fresh sperm quality before chilling. The remaining semen was packed into 0.25 mL plastic straws (IMV Technologies, L'Aigle, France) and sealed with polyvinyl alcohol (IMV Technologies, L'Aigle, France).

The straws were chilled with one of these protocols: (1) in the laboratory, with a programmable water bath (Julabo GmbH, Seelbach, Germany) that chilled the straws from 20 to 4 °C in 90 min (at a rate of −0.18 °C/min) or (2) during transport using a prototype adapted to a car fridge that chilled to 4 °C at an average chilling rate of −0.09 °C/min. One straw from each of the ejaculates was chilled in a similar prototype in the laboratory to perform the analyses of sperm quality. In both cases, the doses were kept at 4 °C until use.

Motility and sperm plasma membrane integrity (**PMI**) were evaluated in fresh and chilled sperm according to the protocols described in detail in Mocé et al. (2020). Briefly, manipulations were performed at room temperature (~22 °C). Motility was determined using a computer-assisted sperm analysis system (ISAS, version 1.0.17, Proiser, Valencia, Spain). Sperm motility was assessed at 37 °C using a 10× negative phase contrast objective on a Nikon Eclipse 90i microscope (Nikon Corporation Instruments Company, IZASA, Barcelona, Spain) connected to the computer through a monochrome Basler A312f video camera (Basler Vision Technologies, Proiser, Paterna, Valencia, Spain). For each sample, the sperm concentration was adjusted with Tris supplemented with bovine serum albumin (0.3%) to 6 × 10^6 sperm/mL and the samples were incubated at 37 °C for 10 min prior to evaluation. Sub-samples of 5 µL were placed on a Makler chamber (Counting Chamber Makler, Sefi-Medical Instruments, Haifa, Israel) prewarmed at 37 °C on a thermal plate, and data from a minimum of 200 sperm from three different fields were collected. Individual sperm tracks were visually assessed to eliminate possible debris and wrong tracks. The following variables were considered in the results (Mocé et al. 2020): percentages of total (**TM**; %) and progressively motile (**PM**; %) sperm, average path velocity (**VAP**; µm/s), curvilinear velocity (**VCL**; µm/s), straight-line velocity (**VSL**; µm/s), straightness index (%), linearity (%), wobble (%), amplitude of the lateral movement of the head (**ALH**; µm) and beat cross frequency (Hz). In addition, a sperm motility index was calculated as (VSL × VCL)/beat cross frequency (Nagata et al., 2018).

The percentage of PMI sperm in each sample was determined using flow cytometry and a dual staining with SYBR-14 and propidium iodide. The samples were analysed after 10 min of incubation using an Epics XL-MCL flow cytometer (Beckman Coulter, IZASA, Barcelona, Spain) with the characteristics described in Mocé et al. (2020). It was equipped with standard optics (a 15-mW 488-nm argon ion laser, Cyonics, Coherent, Santa Clara, CA, USA), and EXPO 2000 software (Coulter Corporation, West Lafayette, IN, USA). The green fluorescence of SYBR-14 was detected using a 550-nm long-pass filter combined with a 525-nm (bandwidth 505–545) band-pass filter (filter 1). The red fluorescence of propidium iodide was detected using a 645-nm long-pass filter combined with a 620-nm (bandwidth 605–635) band-pass filter (filter 3). The settings for the photomultipliers and the compensation values were those described in Mocé et al. (2020). At least 10 000 events per sample were analysed. Any non-DNA containing events (SYBR-14 and propidium iodide negative) were not considered in the calculations, and only the percentages of PMI sperm were included in the results (SYBR-14 positive and propidium iodide negative).

Oestrous synchronisation and artificial insemination

Oestrous was synchronised with a short protocol (Menchaca and Rubianes, 2007) with modifications described in Mocé et al. (2020). Briefly, on day 0, goats were treated with intravaginal pessaries with 30 mg of flugestone acetate (SINCROPART® 30 mg, CEVA Salud Animal, Barcelona, Spain) receiving at the same time an intramuscular injection of 2.5 mg Prostaglandin F2α (Enzaprost® T, CEVA Salud Animal, Barcelona, Spain). The pessaries were removed on day 6, and then, each female received an intramuscular injection of 250 international units (for the AIs performed in autumn or winter) or 300 international units (for the AIs performed during spring or summer) of PMSG (SINCROPART® PMSG 6 000 international units, CEVA Salud Animal, Barcelona, Spain). Only females that had clean pessaries at the moment of removal were included in the study. Cervical AIs were performed on day 8, between 45 and 48 h after PMSG injection. Females were inseminated with chilled semen stored for a maximum of 3 h at 4 °C. Semen was carefully deposited as deep as possible in the cervix avoiding harming the cervix epithelia and semen efflux. Each female was inseminated with one straw (140 × 10^6 total sperm; Mocé et al., 2020), and fertility was determined at parturition, according to the kidding goats. These data were later used as a binary variable (fertility after AI, recorded as kidding 0 or 1) or as kidding rate (calculated as the ratio (number of females kidding/number of females inseminated) × 100).

Table 1
Descriptive analyses for the variables of goat male sperm quality in fresh and chilled samples.

Variables	Fresh sperm					Chilled sperm				
	Mean	Maximum	Minimum	CV (%)	*n*	Mean	Maximum	Minimum	CV (%)	*n*
PMI (%)	67.58	91.80	26.20	19.41	53	55.53	83.91	16.88	25.04	53
TM (%)	79.50	95	60	10.02	60	76.11	94	51	11.58	63
PM (%)	63.77	81	39	14.50	60	60.92	82	36	15.54	63
VCL (µm/s)	134.52	172.42	94.87	12.87	60	142.96	180.10	109.10	10.83	63
VSL (µm/s)	116.99	153.20	81.27	14.50	60	120.60	154.95	87.23	12.79	63
VAP (µm/s)	127.40	165.22	87.85	14.01	60	134.01	172.27	95.53	12.37	63
Linearity (%)	83.51	90.79	74.26	5.25	60	82.80	88.92	70.50	5.35	63
Straightness index (%)	88.69	94.05	81.57	3.18	60	88.16	92.85	80.67	3.36	63
Wobble (%)	92.60	96.35	85.70	3.04	60	92.58	96.04	83.65	3.19	63
ALH (µm)	1.85	2.78	1.47	14.24	60	2.09	3.02	1.60	13.50	63
Beat cross frequency (Hz)	10.69	12.36	8.94	7.59	60	10.91	12.69	9.13	7.05	63
Sperm motility index[1]	1 485.79	2 202.92	787.71	21.89	60	1 592.90	2 291.23	796.99	19.48	63

Abbreviations: n = number of ejaculates; PMI = plasma membrane integrity; TM = total motile sperm; PM = progressively motile sperm; VCL = curvilinear velocity; VSL = straight-line velocity; VAP = average path velocity; ALH = amplitude of the lateral movement of the head.
[1] (VSL × VCL) / Beat cross frequency.

Statistical analysis

Descriptive analyses of the variables were performed (Tables 1 and 2). Pearson's correlation coefficients between fertilities (fertility after AI or kidding rate) and the studied variables were estimated (Tables 3 and 4). Both tables (Tables 3 and 4) show Pearsońs linear correlations. In Table 3, fertility was expressed as kidding rate. In Table 4, fertility was expressed as 0 or 1 and the procedure of choice for estimating correlations between categorical and continuous traits was the point biserial correlation, which is equivalent to the Pearson correlation coefficient when one of the variables is dichotomous. Chi-square test was used to analyse the effect of parturition on fertility (Table 5).

Data were analysed considering the binomial nature of the fertility by logistic regression models that allowed the inclusion of numeric and categorical fixed effects. Moreover, GLM that also allowed the inclusion of random effects were carried out. In logistic regression models, the Wald chi-square test was used as statistical criteria, for testing if the value of the estimated coefficient for a numerical or categorical effect was different than 0 ($P < 0.05$ indicates that it was). In GLM, using a Wald-type test, the significance of each fixed effect was tested with a F-test (type III sums of squares). Moreover, parameters were estimated by maximum likelihood using adaptive Gauss-Hermite quadrature approximation. Several criteria can be used as a measure of goodness of model fit (Akaike information criterion or receiver operating characteristic curves). Receiver operating characteristic curves show a plot of true positives versus the proportion of false positives and the area under the curve. However, model selection using receiver operating characteristic curves does not differ substantially from selection using information criteria. Akaike information criterion is

Table 5
Effect of the parturition order on goat does fertility.

Parturition order	n	Fertility
1	163	72.39 %[a]
2	134	59.70 %[ab]
3	87	50.57 %[b]
>3	111	47.75 %[b]

Abbreviations: n = number of inseminated females.
[1]Fertility expressed as kidding rate (%).
[a,b] Values within a column with different superscripts differ significantly at $P \leq 0.001$ (χ^2 test for testing differences).

Table 2
Descriptive analysis of the variables related with the goat does.

Effects	Mean	Maximum	Minimum	CV (%)	n
Age (d)[1]	1 387.95	4 372	522	45.53	495
Max milk (kg)[2]	590.08	1 396.80	198.30	39.10	495
Milk yield per lactation (kg)[3]	473.22	1 010.62	109.20	31.44	495
Fat-protein (kg)[4]	40.44	80.45	10.47	31.54	495
GV milk (kg)	+31.04	+145.62	−75.13	129.97	292
GV fat (kg)	+1.33	+6.24	−3.91	130.76	292
GV protein (kg)	+1.13	+5.28	−1.90	121.52	292
ICOt	+8.57	+40.82	−20.65	126.91	292

Abbreviations: n = number of inseminations; GV milk = breeding value for milk yield; GV fat = breeding value for fat production; GV protein = breeding value for protein production; ICOt = index combining milk yield and composition.
[1] Age (in days) of the goat doe at the moment of artificial insemination.
[2] Total milk yield obtained in the best lactation from the female.
[3] Averaged milk yield in all the lactations.
[4] Averaged kg of fat and protein produced in all lactations.

Table 3
Correlations[1] between the fertility[2] and quality variables in fresh and in chilled goat male sperm.

Type of sperm	PMI	TM	PM	VCL	VSL	VAP	Linearity[3]	Straightness index[3]	Wobble[3]	ALH	Beat cross frequency[4]	Sperm motility index[5]
Fresh	0.04	0.16	0.19	0.09	0.15	0.13	0.19	0.15	0.20	−0.24	−0.03	0.16
Chilled	−0.07	0.19	0.28	0.03	0.16	0.12	0.29	0.19	0.31	−0.34	−0.25	0.21

Abbreviations: PMI = plasma membrane integrity (%); TM = total motile sperm (%); PM = progressively motile sperm (%); VCL = curvilinear velocity (μm/s); VSL = straight-line velocity (μm/s); VAP = average path velocity (μm/s); ALH = amplitude of the lateral movement of the head (μm).
[1] Correlations (in absolute value) lower than 0.25 were not significantly different from zero ($P > 0.05$).
[2] Fertility expressed as kidding rate (%) per ejaculate.
[3] In %.
[4] Units: Hz.
[5] (VSL × VCL) / Beat cross frequency.

Table 4
Correlations[1] between the fertility[2] and the variables provided by the goat does.

Age[3]	Max milk[4]	Milk yield per lactation[5]	Fat-protein[6]	Parturitions[7]	GV milk	GV fat	GV protein	ICOt
−0.19	−0.11	−0.09	−0.08	−0.20	0.01	−0.01	0.02	0.01

Abbreviations: GV milk = breeding value for milk yield, in kg; GV fat = breeding value for fat production, in kg; GV protein = breeding value for protein production, in kg; ICOt = index combining milk yield and composition.
[1] Correlations (in absolute value) lower than 0.10 were not significantly different from zero ($P > 0.05$).
[2] Fertility: expressed as fertility after artificial insemination (measured as 0 or 1).
[3] Age (in days) of the goat doe at the moment of artificial insemination.
[4] Total milk yield obtained in the best lactation from the female, in kg.
[5] Averaged milk yield in all the lactations, in kg.
[6] Averaged kg of fat and protein produced in all lactations.
[7] Number of parturitions (between 1 and 11).

focused on the fit, and receiver operating characteristic curves are focused on the misclassification. In our study, the Akaike information criterion was selected as a measure of goodness of model fit, being the model adjustment better as the Akaike information criterion value was lower.

The relation of the variables with the *in vivo* fertility was studied in three steps:

- In step 1, fertility after AI was analysed using as independent factors the parameters that the sperm doses presented (PMI, TM, PM, VAP, VSL, VCL, linearity, straightness index, wobble, ALH, beat cross frequency and sperm motility index) in fresh (before chilling) or after chilling to 4 °C (chilled). Session and male effects were also considered. Session effect includes day of ejaculate recovery and other uncontrolled external effects that the data of the same day have in common.
- In step 2, the fertility after AI was analysed including as independent factors the parameters related to the inseminated goats: age, milk yield, max milk, milk yield per lactation, fat-protein, number of parturitions, genetic evaluations (GV milk, GV fat, GV protein and ICOt), session, male and type of refrigeration. Session effect includes season, herd, day of ejaculate recovery and other uncontrolled external effects that the data of the same day have in common.

For finding out the best model to explain fertility using logistic regression, the analyses were performed in the first and second steps following the advices described in Hosmer and Lemeshow (1989). Firstly, univariate analyses were performed to assess any association between fertility and the explanatory variables. Those variables showing $P < 0.25$ were considered as explanatory candidates and used in the next steps. Secondly, all these explanatory candidate variables were included as numeric or fixed effects in a full model and a logistic regression procedure was run with stepwise option in order to obtain the best combinations of variables.

- In step three, the explicative variables of the fertility according to steps 1 and 2 were included as numerical or fixed factors in mixed logistic regression models that also included session and male as random effect because we were interested in their variance and not in the estimated values of these effects. These were joint analyses that included male and female traits at the same time.

These analyses were performed with SAS Statistical Software (Statistical Analysis Systems Institute, 2002).

Results

Step 1. Effects of sperm quality on fertility

The ejaculates presented on average 1.17 mL of volume and 2 790 $\times$ 10^6 sperm/mL of concentration. Global fertility (as kidding percentage) was 59.6%. The average, maximum and minimum values as well as the CV observed for the sperm quality parameters are shown in Table 1. Fresh sperm presented an average quality of 79.5% TM and 67.6% PMI sperm while chilled doses exhibited an average quality of 76.1% TM and 55.5% PMI sperm. The highest CVs were observed for PMI and sperm motility index and the lowest for straightness index and wobble in both fresh and chilled semen.

Table 3 shows the Pearson's coefficients of correlation between fertility and the variables of fresh or chilled sperm quality. Unfortunately, the correlation coefficients between *in vivo* fertility and quality variables of fresh sperm were not significantly different from zero and the correlations with quality variables of chilled sperm were low (below 0.34 in absolute value, negative with ALH or beat cross frequency and positive with PM sperm, linearity and wobble).

Univariate logistic regression models showed that fertility results were explained in part by session effect (or the day of ejaculate recovery, that takes into account the uncontrolled external effects) and the TM and PM sperm of fresh and chilled sperm as well as ALH, linearity, wobble and straightness index of chilled sperm (Table 6).

Next, effects with a $P < 0.25$ in the univariate analysis (the previous ones plus male, sperm motility index and VSL (fresh and chilled) plus VAP, VCL, straightness index and linearity (fresh)) were run in a full model, using the logistic regression procedure with stepwise option. The model for fertility that fitted the best included the session effect and the VAP of fresh sperm (in Table 7, the two models with the best fit are shown). Akaike information criteria are very close because VAP and VCL were highly correlated (r(VAP, VCL) = 0.99).

Step 2. Information provided by the females

The average, maximum and minimum values as well as the CV observed are shown in Table 2. The age of the females was highly variable, ranging from less than 2 until 12 years of age (from 1 to 11 parturitions). There were also important differences between females in milk yield and in all the estimated breeding values being the CVs very high. In Table 4, the Pearson's correlation coefficients between the variables provided by the females and the fertility are shown. As well as for the variables of sperm quality, none of the variables related with the females was highly correlated with fertility (−0.19 with age, −0.20 with parturitions and −0.11 with total milk yield obtained in the best lactation).

Univariate logistic regression models revealed that the variables session (day in which the AI was performed), age of the females, number or code of parturitions and Max milk contributed to explain the fertility (Table 8). Indeed, the lowest the parturition number from a female, the highest the fertility obtained (Table 5). These analyses showed that goats from first kidding were 2.42 times more likely to get pregnant than goats that had kidded four or more times (95% confidence interval of 1.3–4.5). The effects

Table 6
Univariate logistic regression models with one effect considering the information provided by the analyses of quality of fresh and chilled goat buck sperm (n = 63 ejaculates).

Effect	Coefficients (SE) or range (minimum, maximum)	*P*-Wald[1]	Akaike information criterion[2]
TM fresh (%) (n = 60)	0.03 (0.01)	0.01	608.87
PM fresh (%) (n = 60)	0.03 (0.01)	0.01	608.88
Session[3]	range (−1.01, +0.78)	<0.0001	650.89
ALH chilled (μm)	−0.97 (0.35)	0.01	664.19
PM chilled (%)	0.02 (0.01)	0.01	664.47
Linearity chilled (%)	4.74 (2.06)	0.02	666.51
TM chilled (%)	0.02 (0.01)	0.04	667.38
Wobble chilled (%)	6.42 (3.13)	0.04	667.65
Straightness index chilled (%)	5.93 (2.99)	0.05	667.93

Abbreviations: TM = total motile sperm; PM = progressively motile sperm; ALH = amplitude of the lateral movement of the head.

Table 7
Logistic regression models with several fixed effects among the quality variables of fresh and chilled goat male sperm (n = 60 ejaculates).

Effects	Coefficient (SE) or range (minimum, maximum)	*P*-Wald[1]	Akaike information criterion[2]
Model 1			
Session[3]	range (−1.25, +1.04)	<0.0001	587.50
VAP (fresh sperm)	0.03 (0.01)	0.001	
Model 2			
Session[3]	range (−1.27, + 1.05)	<0.0001	587.86
VCL (fresh sperm)	0.03 (0.01)	0.001	

Abbreviations: VAP = average path velocity (μm/s); VCL = curvilinear velocity (μm/s).
[1] Wald χ^2 test for testing the estimated coefficient ($P < 0.05$ indicates that it is different than 0).
[2] Model with only intercept Akaike information criterion = 613.92.
[3] Day of ejaculate recovery (includes the season and other uncontrolled external effects).

Table 8
Univariate logistic regression models with one effect considering the information provided by the goat does (n = 495 inseminations).

Effects	Coefficients (SE) or range (minimum, maximum)	*P*-Wald[1]	Akaike information criterion[2]
Session[3]	range (−1.01, +0.78)	<0.0001	650.89
Parturitions[4]	−0.26 (0.06)	<0.0001	652.63
Age (d)[5]	−0.00064 (0.00015)	<0.0001	653.15
Code parturitions[6]	range (−0.30, +0.64)	0.0002	655.02
Max milk (kg)[7]	−0.00098 (0.00040)	0.014	665.82

[1] Wald χ^2 test for testing the estimated coefficient ($P < 0.05$ indicates that it is different than 0).
[2] Model with only intercept Akaike information criterion = 669.87.
[3] Day of insemination and ejaculate recovery (includes the herd, season and other uncontrolled external effects).
[4] Number of parturitions (between 1 and 11).
[5] Age (in days) of the goat does at the moment of artificial insemination.
[6] Number of parturitions categorised into four levels: 1, 2, 3 and > 3 parturitions.
[7] Total milk yield obtained in the best lactation from the female.

Table 9
Logistic regression models with several fixed effects provided by the goat does inseminated (n = 495 inseminations).

Effects	Coefficients (SE) or range (minimum, maximum)	*P*-Wald[1]	Akaike information criterion[2]
Model 1			
Session[3]	range (+0.74, −0.92)	0.001	642.80
Parturitions[4]	−0.23 (0.08)	0.002	
Model 2			
Session[3]	range (+0.74, −1.03)	0.001	642.74
Age[5]	−0.00060 (0.00019)	0.002	

[1] Wald χ^2 test for testing the estimated coefficient ($P < 0.05$ indicates that it is different than 0).
[2] Model with only intercept Akaike information criterion = 669.87.
[3] Day of insemination and ejaculate recovery (includes the herd, season and other uncontrolled external effects).
[4] Number of parturitions (between 1 and 11).
[5] Age (in days) of the goat does at the moment of artificial insemination.

related with the genetic values did not explain the fertility and the conception did not depend on the refrigeration system either.

Next, effects with a $P < 0.25$ in the univariate analysis (the previous ones plus male, milk per lactation and fat-protein) were run in a full model, using the logistic regression procedure with stepwise option. Table 9 shows the two models with the best adjustments to the variable fertility which included the session and number of parturitions or age of the females.

Step 3. Logistic regressions combining the information provided by the seminal doses and the females

Finally, all the variables that best explained the fertility, coming from sperm laboratory analyses and from the females inseminated, were combined to perform logistic regression analyses. Session effect cannot be under control and that is why it was included as random in the models. Since the data are expressed per inseminated doe, there is a repeated effect of male within session, as well as between sessions. Male effect could be considered as fixed or random, although it was not significant (neither when included as fixed effect nor when included as random), probably due to the screening of the doses that had to meet some minimum quality criteria to be delivered for insemination. Finally, both session and male were included as random effects in the models using different mixed logistic regression models. The best structure of random effects was obtained with a model including just one random effect (session). The five models with the best fit are shown in Table 10. The best adjustment was obtained with a model including just a random effect (session) and the numeric effect age of the females and TM (although similar fit was obtained using the variable PM) or VAP (although similar fit was obtained with the variables VSL or VCL) from fresh sperm.

Table 10
Mixed models of logistic regression with information factors from the goat male sperm quality and from the females inseminated (n = 457 inseminations).

Effects	Coefficient (SE)	$P > F$[1]	Covariance (SE)[2]	Akaike information criterion[3]
Model 1				
Session[4]			0.23 (0.13)	585.10
Age[5]	−0.00051 (0.00019)	0.0074		
TM fresh	0.039 (0.0135)	0.0040		
Model 2				
Session[4]			0.26 (0.16)	588.38
Age[5]	−0.00054 (0.00019)	0.0042		
VAP fresh	0.016 (0.0073)	0.0263		

Abbreviations: TM fresh = total motile sperm in fresh sperm (%) (similar fit was obtained when the variable progressively motility sperm in fresh semen (PM fresh) was included in the model); VAP fresh = average path velocity from fresh sperm (μm/s) (similar fit was obtained when the variables straight-line velocity from fresh sperm (μm/s) (VSL fresh) or curvilinear velocity from fresh sperm (μm/s) (VCL fresh) were included in the model).
[1] Hypothesis test for the significance of the effect ($P < 0.05$ indicates that it is significant).
[2] Estimation of the variance associated to the random effect and its SE.
[3] Model with only session as random effect, Akaike information criterion = 600.10.
[4] Day of insemination and ejaculate recovery (includes the herd, season and other uncontrolled external effects).
[5] Age (in days) of the goat does at the moment of artificial insemination.

Discussion

The fertility prediction of the sperm doses prior to their insemination is one of the priority goals in animal reproduction. However, fertility prediction is elusive due to the complexity of the fertility and the huge number of factors that may have an influence on it (Graham and Mocé, 2005; O'Meara et al., 2007). Since chilled sperm doses are mostly used in goat AI in Spain (Mocé et al., 2020), we focused this study on factors that might affect the fertility of these types of doses in dairy goats.

Considering that fertility depends on the male (quality of the sperm doses) and on the female inseminated, we chose variables from each of them that could impact fertility. The results for *in vitro* sperm quality and milk yield of the goats from our study were in accordance to previous studies with the same breed (Delgado et al., 2017; Mocé et al., 2020). The quality of fresh and chilled sperm was high, and the percentages of total and progressively motile sperm are similar to those reported in previous papers for this species (Xu et al., 2009; Konyali et al., 2013; Santiago-Moreno et al., 2017; Barbas et al., 2018; Mocé et al., 2020; Shadegi et al., 2020). With respect to the kinematic parameters in fresh sperm, our results for some of the parameters (VCL, VAP and ALH) are lower and for the other higher than those reported in Barbas et al. (2018). On the other hand, our kinetic results for chilled sperm are in close accordance with Shadegi et al. (2020) and are in general higher than those reported by other authors (Santiago-Moreno et al., 2017). The differences between studies can be explained by the protocol followed to evaluate the motility (sperm concentration, sperm diluent or cell chamber), the software used or differences between breeds, since all of these factors affect the results (Yeste et al., 2018).

Pearson's correlation coefficients between sperm quality and the fertility were low meaning that none of them are strongly related with fertility rate in a linear way. This low predictive value of individual sperm parameters on conception rate was reported in previous studies (in sheep: O'Meara et al., 2007; in rabbits: Lavara et al., 2005; in pigs: Vyt et al., 2008; Broekhuijse et al., 2012b). This result seems logic, considering the complexity of the fertility and the high number of factors affecting it, as previously exposed. However, we observed that the single variables with higher influence in fertility after running univariate logistic regressions were the percentages of total motile in fresh sperm and progressively motile of fresh and chilled sperm, which is in agreement with previous studies in different species (Lavara et al., 2005; Furstoss et al., 2010; Love, 2011; Fair and Romero-Aguirregomezcorta, 2019). This finding confirms the importance of analysing the motility in the AI doses, since motility is considered as one of the characteristics associated to the sperm fertilising ability (Kathiravan et al., 2011; Nagy et al., 2015) and it is necessary for the sperm to be able to move through the female genital tract and traverse the oocyte vestments (Graham and Mocé, 2005). Besides, some motility parameters of chilled sperm explained some of the variability of the fertility (three parameters derived from velocities (linearity, wobble, straightness index) and ALH). Regression coefficients were positive for the three indexes (linearity, wobble and straightness index), and the sign was negative for ALH. These indexes are higher in regular and linear trajectories that are typical of non-hyperactivated (or forward progressive) sperm (Mortimer, 2000) and are characterised by similar lengths of VSL, VAP and VCL, and little lateral movement of the head (ALH). Although hyperactivation and ALH are acquired during sperm capacitation and are required for the penetration of the oocyte (Broekhuijse et al., 2012b), capacitation must take place at due time inside the female genital tract and not before. Therefore, samples with regular trajectories should be selected for the AIs in dairy goats and, whenever possible, avoiding the delivery of samples with high amplitude, irregular or non-progressive trajectories with high ALH values. However, we would like to highlight that their predictive value for fertility is low, as has been previously exposed. Some of these parameters have been also related with fertility in other species such as rabbits or pigs (Lavara et al., 2005; Vyt et al., 2008), although their sign does not necessarily coincide with our observations. In rabbits, linearity was negatively related (Lavara et al., 2005) and in pigs, ALH was positively related with fertility (Vyt et al., 2008). These controversies between studies can be due to differences between species or storage times, since AI doses remain useful and are usually stored for longer times in these species than in goats. Apart from the parameters provided by the computer-assisted sperm analysis system, we also included the sperm motility index that is calculated from VSL, VCL and beat cross frequency (Nagata et al., 2018). This index is indicative of the intensity of the motility and it apparently showed prognostic value of pregnancy in frozen-thawed microfluidic-sorted bull sperm, being the index lower in the samples more fertile. However, in our study, this index did not show predictive value for the fertility obtained with chilled doses in dairy goats. This controversy between studies can be due to differences between species (bulls *vs.* goats), types of semen (frozen-thawed *vs.* chilled) or treatment received by the semen before AI (microfluidic-sorted or not), among others.

Some of the goat does characteristics had a negative (although very low) impact on fertility. Thus, age and number of parturitions as well as the milk yield obtained in the best lactation had a neg-

ative impact on fertility and they were some of the factors that explained the fertility according to the univariate logistic regression models. The negative effect of female's age on the fertility confirms previous observations where a decline in fertility associated with age was reported (González-Bulnes et al., 2004). One observation to be highlighted is the importance that the number of parturitions has on the AI success since goats from just one kidding were 2.42 times more likely to get pregnant than goats that had kidded four or more times. Therefore, from a practical point of view, technicians should recommend the insemination of females after their first parturition and avoid females with more than one parturition whenever possible. We did not observe any effect of average milk yield per lactation and fat-protein yield on fertility obtained after AI with chilled sperm. However, previous studies in Alpine and Saanen goats reported a low negative effect of milk and fat yield and a strong positive effect of protein yield on fertility of multiparous goats inseminated with frozen-thawed sperm (Furstoss et al., 2015). These differences between studies could be due to differences between breeds, between oestrous synchronisation treatments or between types of sperm (chilled *vs.* frozen-thawed) since freezing-thawing is more damaging and induces more dysfunctions on the sperm than the chilling process (Graham and Mocé, 2005). Recent studies reported that fertility after natural mating was affected in Murciano-Granadina goats only when milk yield at mating was higher than 3 250 mL milk/day (Fernández et al., 2021). In our study, we observed that milk yield obtained in the best lactation had a negative impact on fertility. This could indicate that the fertility could be compromised only in goats with greater yield production. Determining if a threshold value exists over which the fertility is negatively affected after AI with chilled sperm would be of interest for future studies.

As previously exposed, the external factors play a key role in the fertility (Graham and Mocé, 2005). For this reason, the effect of session (including season, herd and other uncontrolled environmental factors such as the management of the females before, during and after AI) on the fertility was expected. When information from the sperm characteristics and from the females inseminated were combined in our study, the best adjustments were obtained with a model that included the session as random and the TM or VAP from fresh sperm and age of the females as fixed effects. Thus, while the age of females presented a negative relation with fertility, TM and VAP presented a positive relation. Sperm needs to be motile in order to travel through the female genital tract and to traverse the oocyte vestments, as has been previously exposed. In addition, VAP is higher in sperm presenting regular and linear trajectories, typical of forward progressive and non-hyperactivated sperm (Mortimer, 2000). From a practical point of view, samples delivered should be selected based on the presence of a high percentage of motile sperm presenting regular trajectories and avoid samples presenting high-amplitude trajectories where the flagellum develops high-amplitude waves in the proximal region that are observed in sperm transitioning to capacitation (Mortimer, 2000). The percentage of total or progressively motile sperm and the velocities VAP or VCL are usually selected in the fertility predictive models (in goats: Furstoss et al., 2010; in rabbits: Lavara et al., 2005; in pigs: Vyt et al., 2008; Broekhuijse et al., 2012b; in rams: Santolaria et al., 2015; in bulls: Nagy et al., 2015). The percentages of motile sperm are always positively related to fertility irrespective of the species (in rabbits: Lavara et al., 2005; in pigs: Vyt et al., 2008; Furstoss et al., 2010; Broekhuijse et al., 2012b). With respect to the velocities, their relation with fertility may be positive (in VAP: Lavara et al. 2005; Nagy et al., 2015; in VCL: Santolaria et al., 2015) or negative (Lavara et al., 2005; Broekhuijse et al., 2012b). However, we would like to indicate that studies are very different among them and the results obtained between them are not directly comparable. Thus, they have been performed in different species that differ in reproductive physiology and types of production. In addition, experimental designs and statistical analyses differ also between studies. Thus, it is not surprising that consensus about the effects that have a higher influence on the fertility does not exist and therefore, whenever possible, the quality of the sperm movement should be evaluated.

We conducted mixed logistic regression models for the data analyses, as we have previously exposed. These analyses are different from those performed in previous studies: multiple regression analyses (in rabbits: Lavara et al., 2005), linear mixed models (in pigs: Broekhuijse et al., 2012b) or logistic regression analyses (in rams: Santolaria et al., 2015). We selected the statistical analyses to conduct based on the characteristics of the effects, the data available and the questions we pretended to answer. We wanted to study the most important effects influencing fertility, both from the point of view of seminal quality evaluated in the laboratory (fresh and refrigerated sperm) and of some characteristics of the inseminated females, as well as the possible effect of the moment of insemination (session effect). Therefore, numerical-covariate effects and categorical effects were needed to be included at the same time in the models. The best choice of statistical procedure for this type of response variables (YES/NO) and various types of effects is logistic regression. Moreover, mixed logistic regression models had to be used for correcting for random effects.

Still, none of the models from our study that combined the variables from sperm and females explained much of the fertility. This limited predictive capacity on field fertility of the models already has been previously reported using different statistical approaches (in pigs: Broekhuijse et al., 2012b; in rams: Santolaria et al., 2015). Although disappointing, several reasons may explain this result. First of all, the aim of AI doses commercialised is to obtain the maximum fertility and, for achieving this, the numbers of sperm per dose are always in excess and above a threshold to minimise risks (Yániz et al., 2018). Therefore, all the males will have reach to the plateau in their corresponding fertility curves and the detection of fertility differences between males will be very difficult (revised by Amann et al., 2018). In addition, a preselection of the ejaculates based on macroscopic and microscopic aspects is also performed and by doing this, we are systematically eliminating naturally occurring variation in male fertility (Taylor et al., 2018), discarding ejaculates with the worst parameters. Moreover, inseminations were performed shortly after semen was collected and probably fertility differences between males due to their resistance to the storage will be observed at longer storage periods (beyond 12–24 h; Leboeuf et al., 2000). And finally, the females to be inseminated must be carefully chosen to maximise the number of females kidding (health and body condition score). These practices minimise the fertility variability but also mask it and hinder the detection of subfertile males. The positive side is that the selection of ejaculates and females for the inseminations are properly performed and therefore, they have few influences on the results obtained. Future studies should take into account these aspects and use lower sperm numbers in the AI doses, a broader selection of sperm quality or longer preservation times in order to detect subfertile males. Our results also highlighted the strong influence of session on fertility. This is an environmental effect very difficult to control because it includes multiple factors, as previously exposed. The prevalence of this factor over the other factors complicates even more the search of a laboratory assay with predictive value. However, efforts must be focused on identifying the extrinsic factors that may have a higher impact on the fertility.

Conclusion

In conclusion, the model with the best adjustment for explaining the fertility results included the session as random effect and

TM or VAP from fresh sperm and age of the females as fixed effects. However, none of the models was satisfactory because they were not very useful to explain the *in vivo* fertility due to the impact of uncontrolled external factors, some of them included in the session effect. From a practical point of view, we recommend to maintain the quality controls for sperm movement, to choose insemination doses presenting high percentages of motile sperm exhibiting regular trajectories and to select the youngest goats (after their first kidding) for insemination.

Ethics approval

Not applicable.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available upon request.

Author ORCIDs

Eva Mocé: https://orcid.org/0000–0003-2895-663X.
María Lorena Mocé: https://orcid.org/0000–0002-9706–6238.
José Bernácer: https://orcid.org/0000–0003-1257–6253.
María del Mar Martínez-Granell: https://orcid.org/0000–0002-8137–7576.
Ernesto A. Gómez (corresponding author): https://orcid.org/0000–0002-8774-420X.

Author contributions

Eva Mocé: Conceptualization, Investigation, Resources, Writing – Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition. **María Lorena Mocé:** Formal analysis, Data Curation, Writing – Original Draft, Writing - Review & Editing, Supervision, Funding acquisition. **Salvador A. Lozano-Palazón:** Investigation, Resources, Data Curation, Writing - Review & Editing. **José Bernacer:** Investigation, Writing - Review & Editing. **María del Mar Martínez-Granell:** Investigation, Writing - Review & Editing. **Carolina Esteve:** Investigation, Writing - Review & Editing. **Francisco Bernat:** Investigation, Writing - Review & Editing. **Sócrates J. Contreras:** Investigation, Data Curation, Writing - Review & Editing. **Irene Villalba:** Investigation, Writing - Review & Editing. **Ernesto A. Gómez**: Conceptualization, Formal analysis, Resources, Data Curation, Writing – Original Draft, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of interest.

None.

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References

Amann, R.P., Saacke, R.G., Barbato, G.F., Waberski, D., 2018. Measuring male-to-male differences in fertility or effects of semen treatments. Annual Review of Animal Biosciences 6, 255–286.
Barbas, J.P., Leahy, T., Horta, A.E., García-Herreros, M., 2018. Sperm kinematics and subpopulational responses during the cryopreservation process in caprine ejaculates. Cryobiology 82, 137–147.
Barth, A.D., 2018. Review: The use of bull breeding soundness evaluation to identify subfertile and infertile bulls. Animal 12, 158–164.
Belanche, A., Martín-García, A.I., Fernández-Álvarez, J., Pleguezuelos, J., Mantecón, A. R., Yáñez-Ruiz, D.R., 2019. Optimizing management of dairy goat farms through individual animal data interpretation: A case study of smart farming in Spain. Agricultural Systems 173, 27–38.
Berry, D.P., Wall, E., Pryce, J.E., 2014. Genetics and genomics of reproductive performance in dairy and beef cattle. Animal 8, 105–121.
Broekhuijse, M.L.W.J., Feitsma, H., Gadella, B.M., 2012a. Artificial insemination in pigs: predicting male fertility. Veterinary Quarterly 32, 151–157.
Broekhuijse, M.L.W.J., Sostaric, E., Feitsma, H., Gadella, B.M., 2012b. Application of computer-assisted semen analysis to explain variations in pig fertility. Journal of Animal Science 90, 779–789.
Delgado, J.V., León, J.M., Gómez, M., Castillo, J., Lozano, S., Fernández, J., 2017. Programa de mejora genética en la raza murciano-granadina [Breeding program in Murciano-Granadina goat breed]. Tierras 20, 51–55.
Fair, S., Romero-Aguirregomezcorta, J., 2019. Implications of boar sperm kinematics and rheotaxis for fertility after preservation. Theriogenology 137, 15–22.
Fernández, N., Beltrán, M.C., Romero, G., Roca, M.A., Rodríguez, M., Balasch, S., 2021. Pointing out some issues regarding reproduction management in Murciano-Granadina goats. Animals 11, 1781.
Fetrow, J., Nordlund, K.V., Norman, H.D., 2006. Invited review: Culling: Nomenclature, definitions, and recommendations. Journal of Dairy Science 89, 1896–1905.
Furstoss, V., Borderes, F., Forgerit, Y., Guillouet, P., Leboeuf, B., 2010. The value of the percentage of motile sperm in predicting a significant portion of the fertility variation of frozen-thawed buck semen. Theriogenology 74, 1197–1206.
Furstoss, V., David, I., Fatet, A., Boissard, K., Clément, V., Bodin, L., 2015. Genetic and non-genetic factors related to the success of artificial insemination in dairy goats. Animal 9, 1935–1942.
Graham, J.K., Mocé, E., 2005. Fertility evaluation of frozen-thawed semen. Theriogenology 64, 492–504.
González-Bulnes, A., Baird, D.T., Campbell, B.K., Cocero, M.J., García-García, R.M., Inskeep, E.K., López-Sebastián, A., McNeilly, A.S., Santiago-Moreno, J.S., Souza, C. J.H., Veiga-López, A., 2004. Multiple factors affecting the efficiency of multiple ovulation and embryo transfer in sheep and goats. Reproduction, Fertility and Development 16, 421–435.
Hadley, G.L., Wolf, C.A., Harsh, S.B., 2006. Dairy cattle culling patterns, explanations, and implications. Journal of Dairy Science 89, 2286–2296.
Hosmer, D.W., Lemeshow, S., 1989. Applied Logistic Regression. John Wiley & Sons Inc., New York, NY, USA.
Kathiravan, P., Kalatharan, J., Karthikeya, G., Rengarajan, K., Kadirvel, G., 2011. Objective sperm motion analysis to assess dairy bull fertility using computer-aided system - A review. Reproduction in Domestic Animals 46, 165–172.
Konyali, C., Tomás, C., Blanch, E., Gómez, E.A., Graham, J.K., Mocé, E., 2013. Optimizing conditions for treating goat semen with cholesterol-loaded cyclodextrins prior to freezing to improve cryosurvival. Cryobiology 67, 124–131.
Kumaresan, A., Das Gupta, M., Datta, T.K., Morrell, J.M., 2020. Sperm DNA integrity and male fertility in farm animals: a review. Frontiers in Veterinary Science 7, 321.
Lavara, R., Mocé, E., Lavara, F., Viudes de Castro, M.P., Vicente, J.S., 2005. Do parameters of seminal quality correlate with the results of on-farm inseminations in rabbits? Theriogenology 64, 1130–1141.
Leboeuf, B., Restall, B., Salamon, S., 2000. Production and storage of goat semen for artificial insemination. Animal Reproduction Science 62, 113–141.
Lonergan, P., Fair, T., Forde, N., Rizos, D., 2016. Embryo development in dairy cattle. Theriogenology 86, 270–277.

Love, C.C., 2011. Relationship between sperm motility, morphology and the fertility of stallions. Theriogenology 76, 547–557.
Lucy, M.C., 2001. Reproductive loss in high-producing dairy cattle: where will it end? Journal of Dairy Science 84, 1277–1293.
Ma, L., Sonstegard, T.S., Cole, J.B., VanTassell, C.P., Wiggans, G.R., Crooker, B.B.A., Tan, C., Prakapenka, D., Liu, G.E., Da, Y., 2019. Genome changes due to artificial selection in U.S: Holstein cattle. BMC Genomics 20, 128.
Malher, X., Seegers, H., Beaudeau, F., 2001. Culling and mortality in large dairy goat herds managed under intensive conditions in western France. Livestock Production Science 71, 75–86.
Menchaca, A., Rubianes, E., 2007. Pregnancy rate obtained with short-term protocol for timed artificial insemination in goats. Reproduction in Domestic Animals 42, 590–593.
Mendoza, N., Casao, A., Domingo, J., Quintín, F., Laviña, A., Fantova, E., Cebrián-Pérez, J.A., Muiño-Blanco, T., Pérez-Pe, R., 2021. Influence of non-conventional sperm quality parameters on field fertility in ovine. Frontiers in Veterinary Science 8, 650572.
Mocé, E., Lozano-Palazón, S.A., Martínez-Granell, M.A., Mocé, M.L., Gómez, E.A., 2020. Effect of the refrigeration system on *in vitro* quality and *in vivo* fertility of goat buck sperm. Animals 10, 2399.
Mortimer, S.T., 2000. CASA-Practical aspects. Journal of Andrology 21, 515–524.
Nagata, M.P.B., Endo, K., Ogata, K., Yamanaka, K., Egashira, J., Katafuchi, N., Yamanouchi, T., Matsuda, H., Goto, Y., Sakatani, M., Hojo, T., Nishizono, H., Yotsushima, K., Takenouchi, N., Hashiyada, Y., Yamashita, K., 2018. Live births from artificial insemination of microfluidic-sorted bovine spermatozoa characterized by trajectories correlated with fertility. Proceedings of the National Academy of Sciences USA 115, E3087–E3096.
Nagy, A., Polichronopoulos, T., Gáspárdy, A., Solti, L., Cseh, S., 2015. Correlation between bull fertility and sperm velocity parameters generated by computer-assisted semen analysis. Acta Veterinaria Hungarica 63, 370–381.
O'Meara, C.M., Donovan, A., Hanrahan, J.P., Duffy, P., Fair, S., Evans, A.C.O., Lonergan, P., 2007. Resuspending ram spermatozoa in seminal plasma after cryopreservation does not improve pregnancy rate in cervically inseminated ewes. Theriogenology 67, 1262–1268.
Rajala-Schultz, P.J., Frazer, G.S., 2003. Reproductive performance in Ohio dairy herds in the 1990s. Animal Reproduction Science 76, 127–142.
Real Decreto 53/2013 de 1 de febrero por el que se establecen las normas básicas aplicables para la protección de los animales utilizados en experimentación y otros fines científicos, incluyendo la docencia [Royal Decree 53/2013 of 1 February, which establishes the basic rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching]. Retrieved on 10 March 2021 from https://www.boe.es/buscar/pdf/2013/BOE-A-2013-1337-consolidado.pdf.
Royal, M.D., Darwash, A.O., Flint, A.P.F., Webb, R., Woollians, J.A., Lamming, G.E., 2000. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Animal Science 70, 487–501.
Santiago-Moreno, J., Esteso, M.C., Castaño, C., Toledano-Díaz, A., Delgadillo, J.A., López-Sebastián, A., 2017. Seminal plasma removal by density-gradient centrifugation is superior for goat sperm preservation compared with classical sperm washing. Animal Reproduction Science 181, 141–150.
Santolaria, P., Vicente-Fiel, S., Palacín, I., Fantova, E., Blasco, M.E., Silvestre, M.A., Yániz, J.L., 2015. Predictive capacity of sperm quality parameters and sperm subpopulations on field fertility after artificial insemination in sheep. Animal Reproduction Science 163, 82–88.
Institute, SAS, 2002. SAS user's guide, version 9.00. SAS. Institute Inc., Cary, NC, USA.
Shadegi, S., del Gallego, R., García-Colomer, B., Gómez, E.A., Yániz, J.L., Gosálvez, J., López-Fernández, C., Silvestre, M.A., 2020. Effect of sperm concentration and storage temperature on goat spermatozoa during liquid storage. Biology 9, 300.
Silvestre, M.A., Salvador, I., Sánchez, J.P., Gómez, E.A., 2004. Effect of changing female stimulus on intensive semen collection in young Murciano-Granadina male goats. Journal of Animal Science 82, 1641–1645.
Taylor, J.F., Schnabel, R.D., Sutovsky, P., 2018. Review: Genomics of bull fertility. Animal 12, 172–183.
Vyt, P., Maes, D., Quinten, C., Rijsselaere, T., Deley, W., Aarts, M., de Kruif, A., Van Soom, A., 2008. Detailed motility evaluation of boar semen and its predictive value for reproductive performance in sows. Vlaams Diersgeneeskundig Tijdschrift 77, 291–298.
Xu, C.L., Zhou, J.B., Zhao, B.T., Lan, G.C., Luo, M.J., Chang, Z.L., Sui, H.S., Tan, J.H., 2009. Liquid storage of goat semen in chemically defined extenders. Reproduction in Domestic Animals 44, 771–778.
Yániz, J., Silvestre, M.A., Santolaria, P., Soler, C., 2018. CASA-Mot in mammals: an update. Reproduction, Fertility and Development 30, 799–809.
Yeste, M., Bonet, S., Rodríguez-Gil, J.E., Rivera del Álamo, M.M., 2018. Evaluation of sperm motility with CASA-Mot: which factors may influence our measurements? Reproduction, Fertility and Development 30, 789–798.