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Meta-analysis of the efficacy of melatonin implants for improving reproductive performance in sheep

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Abstract

To evaluate the efficacy of melatonin implants on increasing the production of lambs, a meta-analysis was performed based on the results of 139 experiments carried out in Spain and published in 56 scientific publications or conference proceedings. To assess the effects on fertility, the relative risk (RR) was calculated and, in the case of prolificacy and fecundity, the analysis was based on raw mean difference (D) and the standardized difference between means (d) and their corresponding confidence intervals. In addition, the experiments were stratified by the productive aptitude of the flocks, breed, time of year, and age. For fertility, the mean RR was 1.29, which indicated that the treatment with melatonin increased significantly (by 29%) the probability of pregnancy. For prolificacy, overall D was 0.08 ($d = 0.9089$); *i.e.,* the groups treated with melatonin increased their litter size by 0.08 lambs/lambing relative to the control groups. Ultimately, treatment with melatonin increased fecundity significantly (0.25 extra lambs/ewe treated) ($d = 6.3188$), and this trend was evident in all of the stratified datasets. In all cases, there was significant ($p < 0.001$) heterogeneity and bias. In conclusion, in Spain, melatonin implants had a significant effect on the probability that a ewe became pregnant, increased the number of lambs born per lambing, which ultimately increased the number of lambs born per treated ewe. In addition to confirm the results of previous descriptive reviews in the scientific literature, the meta-analysis made use of a statistical tool that synthesizes and estimates more precisely the effect of this hormone on sheep reproduction.

Additional key words: literature review; ovine reproduction; systematic review.

Resumen

Meta-análisis de la eficacia de los implantes de melatonina para incrementar los rendimientos reproductivos de la especie ovina

Se ha realizado un meta-análisis sobre la eficacia del uso de implantes de melatonina en ovino en España, analizando los resultados de 139 experiencias publicadas en 56 publicaciones o comunicaciones. Para la fertilidad se han calculado los riesgos relativos (RR) y para la prolificidad y fecundidad se han calculado la diferencia no estandarizada o bruta entre medias (D) y la diferencia estandarizada entre medias (d). Los resultados se han estratificado por aptitud productiva de los rebaños, raza, época del año y edad. Se han calculado los estadísticos de heterogeneidad y el posible sesgo entre publicaciones. Para la fertilidad, el RR medio fue 1,29, lo que significa que la melatonina incrementó de manera significativa las posibilidades de parto un 29%. Para la prolificidad, la D global fue 0,08 ($d = 0{,}9089$), es decir, los grupos tratados incrementaron su prolificidad 0,08 corderos/parto. El tratamiento con melatonina aumentó de manera significativa la fecundidad, con 0,25 corderos extra producidos/oveja tratada ($d = 6{,}3188$). En todos los casos, la heterogeneidad y el sesgo calculados fueron significativos ($p < 0{,}001$). En conclusión, el meta-análisis realizado ha revelado un efecto significativo de la melatonina en España sobre la posibilidad de que una oveja quede gestante, además de elevar el número de corderos nacidos por parto, lo que al final incrementa el número de corderos nacidos por oveja tratada. Esto no ha hecho sino confirmar lo revisado previamente en la literatura de manera narrativa, mediante una herramienta estadística, que sintetiza y estima de manera más precisa el efecto de esta hormona.

Palabras clave adicionales: reproducción de ovinos; revisión bibliográfica; revisión sistemática.

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Abbreviations used: CI (confidence interval); d (standardized difference between means); D (raw mean difference); RR (relative risk).

Introduction

The practical use of melatonin to improve the reproductive variables in sheep began in the 1980s in the UK, Australia, and New Zealand resulting in an important change in the commercial use of hormones in this species. Eventually, the route of application adopted was subcutaneous mini-implants (2 × 4 mm, 18 mg melatonin) applied at the base of the ear (Regulin, CEVA Animal Health). In 2000, it was commercialized in Spain (Melovine, CEVA Santè Animale). Before rigorous studies were initiated in this country, the application protocol for melatonin implants was based on their use in Northern Europe, especially in the UK and France. Due to the high sexual seasonality of the sheep breeds at those latitudes, melatonin implants are used in June, when Mediterranean breeds practically already have initiated their period of sexual activity. One of the principal contributions of our research group has been to describe the use of melatonin in our native breeds already from the winter solstice, extending its application until the spring equinox and well into May (Forcada *et al.*, 2002; Zúñiga *et al.,* 2002; Abecia *et al.,* 2007). Furthermore, the treatment has demonstrated to be effective even in the months of sexual activity at Southern latitudes, which has led to an in-depth examination of the temporal protocols for the application of this hormone in all of the Mediterranean breeds of sheep. Thus, the information obtained has been invaluable to the sheep producers in Mediterranean countries where melatonin is available (Portugal, Italy and Greece).

An earlier review of the reproductive results of commercial sheep operations in Spain following the implementation of melatonin implants (Abecia *et al.*, 2003) demonstrated the efficacy of the treatment in the sheep breeds (13 breeds on 78 farms) reared in Spain, with a mean lamb production improvement of 40%. Factors that might influence the effectiveness of the treatment such as the time of year in which treatment took place, the production system, and the physiological state of the animal have been examined in a variety of studies. It has been confirmed (Abecia *et al.*, 2007) that the efficacy of the treatment within each breed varied as a function of the production system and the timing of the treatment. For instance, melatonin treatments improved the reproductive variables in Rasa Aragonesa ewes that were reared under one of three production systems (intensive, semi-extensive, extensive), obtaining better results in the extensive systems (Palacín *et al.*, 2006). As well, the efficacy of the implants can be greater in years of exceptional drought and in management systems closely tied to the consumption of pasture, where the animals can suffer nutritional deprivations (Arrébola *et al.*, 2009). In addition, the treatment is a good option in extensive production farms that are characterized by limited manpower because the protocol involves only one manipulation of the animals. With respect to the physiological state of the animal, while following the commercial protocol treatment implants would be placed around day 45 after parturition, the reproductive results were greater when the implants were inserted around the time of lambing (Abecia *et al.*, 2002).

Spain has reached the highest market share of the melatonin implants for sheep in the world, with more than 400,000 treatments applied in the ovine population in 2009, which is about 16 million heads; it means that 1 out of 40 ewes in Spain have been treated with melatonin. After 10 yr of the use of this hormone, there was a need for a further review of the results obtained by research groups. The number of scientific publications on the subject has dramatically increased in recent years. Given the vast and varied amount of original articles on a variety of breeds, production systems, physiological states, there is a need for a through critical review.

Any review of the scientific literature should include the compilation, analysis, synthesis, and discussion of the information published on the subject in question (Garfield, 1987). Traditional (qualitative) reviews are characterized by the absence of any statistical or mathematical analysis and are limited to describing narratively a large amount of information with the objective of finding some theory or connection between the results of the studies reviewed. When the number of scientific articles is high, the interpretation of the results in that type of review can be incorrect and inaccurate because a large amount of information can lead to a more or less subjective bias on the part of the reviewer toward the most relevant publications. Systematic reviews attempt to address those limitations by establishing a rigorous, formal methodology. They describe explicitly the methodological process followed, focusing on the sources of information, the criteria for inclusion, and the method of analysis and synthesis used, which leads to more comprehensive scientific reviews, limiting further the biases in the studies and in interpreting the results. The incorporation of meta-analysis as a tool for scientific review, especially in clinical epidemiology, has meant an improvement in the scientific rigor of research reviews because it incorporates statistical analysis in the methodology of systematic reviews.

The meta-analysis was proposed in 1976 by Glass (1976), who defined it as "the statistical analysis of a large collection of results derived from individual studies in order to integrate the findings". It allows a review of both qualitative (descriptive) and quantitative (numerical) statistical data from reports and published studies, which seeks to address a common objective. The result is the combined evaluation of the magnitude and direction of the effect suggested by a suite of more-or-less extensive studies, an effect that individually might not be appreciated. For that reason, one of the advantages of meta-analysis is the possibility of including studies that have a small sample size because the technique allows the prediction of the effect of each test against the size of the study. On the other hand, a critical aspect of the methodology is the possibility of publication bias because studies that obtain statistically significant results are more likely to be published than are those with non-significant results. In any case, the meta-analysis appears to be an efficient technique for scientific review more efficient because it combines statistically the results to obtain a single overall value that provides a more accurate assessment and interpretation of the effect.

The objective of this review was to perform a meta-analysis of the existing literature on the effect of the use of melatonin implants to improve the reproductive performance of sheep, under the null hypothesis that the reproductive variables fertility, prolificacy and fecundity were not affected by treatment with melatonin. We have selected Spain for the enormous amount of experience collected controlling sexual activity in sheep and because it represents the Mediterranean countries, where this treatment has been widely applied. Previous systematic reviews on the use of melatonin to treat sleep disorders in humans (Brzezinski *et al.*, 2005; Buscemi *et al.*, 2006) or on the role of this hormone in cancer (Mills *et al.*, 2005) have been published.

Material and methods

Search for articles

The literature review of the research focused on experiments on the use of subcutaneous melatonin implants in the sheep and its effects on reproductive variables. The search for publications was made in national and international scientific and technical journals, as well as publications from national or international conferences. We used the ISI database Web of Knowledge to obtain papers referenced in the database, although it was completed with an electronic search of other studies not referenced therein. In addition, the collections from the library at the University of Zaragoza were consulted to look for studies referenced in others not available in electronic format.

Criteria for inclusion in the meta-analysis

Several criteria were used to define whether an experiment was included in the meta-analysis; specifically, (1) that the experiments were performed in Spain, (2) the experimental design of the study included a group treated with melatonin implants and a control group that did not receive the treatment, and included the number of animals in each group, (3) the study included information on the number of pregnancies or fertility (number of pregnancies/number of ewes in the group) to make a 2×2 table (pregnancy or non pregnancy *vs.* treated or not treated), or (4) the study presented average values and at least one measurement of dispersion (standard deviation or standard error) of the reproductive index of prolificacy, or (5) fecundity. Failure to meet criteria (1) and (2) was sufficient for a study to be excluded from the analysis, although the other inclusion criteria were considered study variables since in some cases, not all of the three reproductive variables were presented in the same study.

Preparing the list of studies

After gathering all of the information, it was tabulated in Excel, referenced by the primary author's last name followed by *et al.* and the year of publication, which was the variable to order the studies (from the oldest to the most recent). Experiments included in the analysis were published over an 18-yr period (between 1991 and 2009). Several publications included more than one experiment (*e.g.,* different breeds, localities, farms, years), and each was considered individually. The factors for grouping the experiments were the productive aptitude of the sheep (meat, milk, or mixed-use), the breed (Rasa Aragonesa, Assaf, or Merino), time of year (anoestrus or reproductive season) and age of the animals (adults or ewe-lambs).

Statistical analyses

In the case of dichotomous variables such as fertility (pregnancy or not), the overall estimate of the effect of the use of melatonin implants was performed by measuring the relative risk (RR) response in the treated group in relation to the control group and its corresponding confidence interval (CI). In that case, the risk is interpreted as the increase in the probability that an animal treated with melatonin achieves pregnancy. In the case of continuous variables such as prolificacy (number of lambs born/lambing) and fecundity (number of lambs born/100 ewes), the raw mean difference (*D*) and the standardized difference between means (*d*), and their corresponding CI, were calculated. Based on the low statistical power of the test to detect any heterogeneity present, evidence of an effect was rejected when a *p*-value was < 0.20 and, therefore, the CI were calculated at 80%. To illustrate the relative strength of treatment effects, forest plots for RR (fertility) and *d* (prolificacy and fecundity) have been drawn.

The use of *D* in a meta-analysis, expressed as the difference between the mean of the group treated with melatonin and the control group, is valid when, as in this study, all of the studies are expressed on the same scale (number of lambs) and the methods in all of them are identical. On the other hand, *d* (Cohen, 1988) is a measure that leads to the same scale the degree of separation between the means of various studies; *d* is calculated by dividing the difference between the means by the mean standard deviation of the control group, which results in a measure of the difference expressed in terms of the deviation between groups. For example, a *d* of 0.25 indicates that the two means are separated by a quarter of the standard deviation. In addition, that statistic allows a comparison of the effects between the groups in question (*e.g.,* reproductive aptitude, breed, and season).

Heterogeneity was evaluated using the Q-test of Dersimonian. Due to the low power of the existing statistical tests, these were supplemented with Galbraith and L'Abbé plots. The Galbraith plot represents the precision of each study relative to the standardized effect. In addition, it represents the regression line fitted to these points and the confidence interval. The studies that fell outside of the confidence interval were those that contributed most to heterogeneity. The L'Abbé plot represents the event rate in the treatment group compared to the rate of the control group; therefore, this plot only is used for binary response variables. Since all of the studies showed a significant *p*-value of heterogeneity, the random-effect model was applied.

The studies were subjected to a sensitivity analysis, which assesses the influence of each of the studies on the overall estimate of the effect and, therefore, the stability of the overall measurement. The sensitivity analysis was performed by repeating the meta-analysis as many times as there were studies, each time omitting a study.

In the evaluation of publication bias or other biases (generated by the methodological quantity or quality of the studies), the regression asymmetry test of Egger and the correlation test of Begg (adjusted rank correlation method) was applied. An overall meta-analysis was performed and one for each of the reproductive aptitudes (meat, dairy, or mixed-use), three breeds (Rasa Aragonesa, Assaf, and Merino), season (anoestrus *vs.* reproductive season), and age (adults *vs.* ewe-lambs). The meta-analysis was performed using Epidat 3.1. (2006).

Results

Qualitative meta-analysis

In total, 139 experiments in 56 scientific publications were deemed to have potentially relevant results; however, based on the inclusion criteria, 124 experiments (in 46 publications, Table 1) were selected for a more thorough evaluation. Of those, 77 (62%) involved flocks managed for meat, 31 (25%) used dairy breeds, and 16 (13%) were based on mixed meat-dairy production systems. Thirty-six (29%) of the studies were performed on Rasa Aragonesa breed, 28 (23%) on Assaf and crosses, and 34 (27%) on Merino and crosses. Another 26 (21%) studies involved other breeds, but they could not be included in the meta-analysis of the effects of melatonin on individual breeds because of the small number of studies (*e.g.,* 1 Segureña, 3 Churra), but were included in the overall meta-analysis. In addition, in 105 experiments (85%) the subjects were adults; ewe-lambs were subjects in the remainder of the experiments.

Among the publications, 7 of 46 (15%) were published in international journals, two (4%), were published in

Table 1. Sources (in chronological order) used in the meta-analysis of experiments that tested the effect of melatonin treatments on the reproductive variables of Spanish sheep. Productive aptitude and breed are indicated

Reference	Aptitud, breed	Reference	Aptitud, breed	Reference	Aptitud, breed
1. Folch *et al.*, 1991a	Meat RA	43. Forcada *et al.*, 2002	Meat RA	85. Palacín *et al.*, 2006	Meat RA
2. Folch *et al.*, 1991a	Meat RA	44. Gascón *et al.*, 2002	Mixed X×LA	86. Palacín *et al.*, 2006	Meat RA
3. Folch *et al.*, 1991a	Meat RA	45. Martín *et al.*, 2002a	Meat ME	87. Palacín *et al.*, 2006	Meat RA
4. Folch *et al.*, 1991b	Meat RA	46. Martín *et al.*, 2002b	Meat RI×CA	88. Palacios *et al.*, 2006	Meat LA
5. Folch *et al.*, 1991b	Meat MA	47. Martín *et al.*, 2002b	Meat RI×CA	89. Palacios *et al.*, 2006	Dairy AS
6. López and Inskeep, 1991	Meat RA	48. Mejías *et al.*, 2002	Meat ME×SE	90. Palacios *et al.*, 2006	Dairy AS
7. López and Inskeep, 1991	Meat RA	49. Palacios *et al.*, 2002	Dairy AS	91. Palacios *et al.*, 2006	Mixed CH
8. López and Inskeep, 1991	Meat T	50. Palacios *et al.*, 2002	DairyAS	92. Palacios *et al.*, 2006	Mixed MA
9. López and Inskeep, 1991	Mixed CH	51. Palacios *et al.*, 2002	Mixed CH	93. Requejo *et al.*, 2006	Dairy LA
10. López and Inskeep, 1991	Mixed CH	52. Palacios *et al.*, 2002	Mixed CA	94. Abecia *et al.*, 2007	Meat RA
11. Gómez *et al.*, 1995	Meat ME	53. Valares, *et al.*, 2002	Meat RA	95. Abecia *et al.*, 2007	Meat RA
12. Gómez *et al.*, 1995	Meat ME	54. Gascón *et al.*, 2003	Mixed X×LA	96. Abecia *et al.*, 2007	Meat RA
13. Gómez *et al.*, 1995	Meat RA	55. Gómez *et al.*, 2003	Dairy LA	97. Abecia *et al.*, 2007	Meat RA
14. Forcada *et al.*, 1998	Meat RA	56. Martín, 2003	Meat ME	98. Abecia *et al.*, 2007	Meat ME
15. Forcada *et al.*, 1998	Meat RA	57. Palacios *et al.*, 2003	Dairy AS	99. Abecia *et al.*, 2007	Meat ME
16. Forcada *et al.*, 1998	Meat RA	58. Palacios *et al.*, 2003	Dairy AS	100. Abecia *et al.*, 2007	Meat ME
17. Forcada *et al.*, 1998	Meat RA	59. Palacios *et al.*, 2003	Dairy AS	101. Abecia *et al.*, 2007	Meat ME
18. Forcada *et al.*, 1998	Meat RA	60. Palacios *et al.*, 2003	Dairy AS	102. Abecia *et al.*, 2007	Dairy AS
19. Forcada *et al.*, 1998	Meat RA	61. Palacios *et al.*, 2003	Dairy AS	103. Abecia *et al.*, 2007	Dairy AS
20. Ciudad *et al.*, 1999	Meat RA	62. Palacios *et al.*, 2003	Dairy AS	104. Abecia *et al.*, 2007	Dairy AS
21. Folch and Alabart, 1999	Meat RA	63. Palacios *et al.*, 2003	Dairy AS	105. Abecia *et al.*, 2007	Dairy AS
22. Folch and Alabart, 1999	Meat RA	64. Pontes *et al.*, 2003	Meat MA	106. López and Sánchez, 2007	Mixed ME
23. Forcada *et al.*, 1999	Meat RA	65. Sánchez *et al.*, 2003	Meat ME×FL	107. López and Sánchez, 2007	Mixed ME
24. Forcada *et al.*, 1999	Meat RA	66. Sánchez *et al.*, 2003	Meat ME×FL	108. López and Sánchez, 2007	Mixed ME
25. Legaz *et al.*, 2000	Dairy MA×AS	67. Sánchez *et al.*, 2003	Meat ME×FL	109. Tamayo *et al.*, 2007	Dairy AW
26. Legaz *et al.*, 2000	Dairy MA×AS	68. Sánchez *et al.*, 2003	Meat ME×FL	110. Tamayo *et al.*, 2007	Dairy AS
27. García *et al.*, 2001	Meat ME	69. Sánchez *et al.*, 2003	Meat ME×FL	111. Tamayo *et al.*, 2007	Dairy AS×CH
28. García *et al.*, 2001	Meat ME×FL	70. Sánchez *et al.*, 2003	Meat ME×FL	112. Tamayo *et al.*, 2007	Dairy AW
29. García *et al.*, 2001	Meat ME×CHA	71. Santander *et al.*, 2003	Meat RA	113. Tamayo *et al.*, 2007	Dairy AS×CH
30. García *et al.*, 2001	Meat ME×CHA	72. Gutiérrez *et al.*, 2004	Mixed CH	114. Tamayo *et al.*, 2007	Dairy AW
31. García *et al.*, 2001	Meat MP	73. Gutiérrez *et al.*, 2004	Mixed CH	115. Arrebola *et al.*, 2008	Meat ME
32. Maqueda *et al.*, 2001	Meat ME	74. Pontes *et al.*, 2004	Meat MA	116. Arrébola *et al.*, 2008	Meat ME
33. Palacios *et al.*, 2001	DairyAS	75. Ramírez *et al.*, 2004	Mixed MA	117. Arrébola *et al.*, 2008	Meat ME
34. Puntas *et al.*, 2001	Meat SE	76. Argote *et al.*, 2005	Meat RA	118. Arrébola *et al.*, 2008	Meat ME
35. Puntas *et al.*, 2001	Meat SE	77. Argote *et al.*, 2005	Meat RA	119. Arrébola et al,, 2008	Meat ME
36. Abecia *et al.*, 2002	Meat RA	78. Argote *et al.*, 2005	Meat RA	120. Arrébola *et al.*, 2008	Meat ME
37. Abecia *et al.*, 2002	Meat RA	79. Arrébola *et al.*, 2005	Meat ME	121. Arrébola *et al.*, 2009	Meat ME
38. Abecia *et al.*, 2002	Meat RA	80. Palacios *et al.*, 2005	Dairy AS	122. Arrébola *et al.*, 2009	Meat ME
39. Abecia *et al.*, 2002	Meat RA	81. Requejo *et al.*, 2005	Dairy AS	123. Arrébola *et al.*, 2009	Meat ME
40. Carbonero *et al.*, 2002	Mixed CR	82. Gómez *et al.*, 2006	Dairy MA	124. Arrébola *et al.*, 2009	Meat ME
41. Carbonero *et al.*, 2002	Mixed CR	83. Legaz *et al.*, 2006	Dairy AS		
42. Forcada *et al.*, 2002	Meat RA	84. Legaz *et al.*, 2006	Dairy AS		

AS: Assaf. AW: Awassi. CA: Castellana. CH: Churra. CHA: Charmoise. FL: Fleischschaf. LA: Lacaune. MA: Manchega. ME: Merino. MP: Merino Precoz. RA: Rasa Aragonesa. RI: Ripollesa. T: Talaverana. X: Xisqueta.

Spanish technical journals, and 37 were presented in the proceedings of national conferences (80%). Of the latter, all but two of the papers were presented at several conferences of the Congress of the Spanish Society of Sheep and Goat Production (SEOC).

Quantitative meta-analysis

Forest-plots of relative risk and the standardized mean difference are presented in Figure 1. It reflects the results of the meta-analysis and consists of two

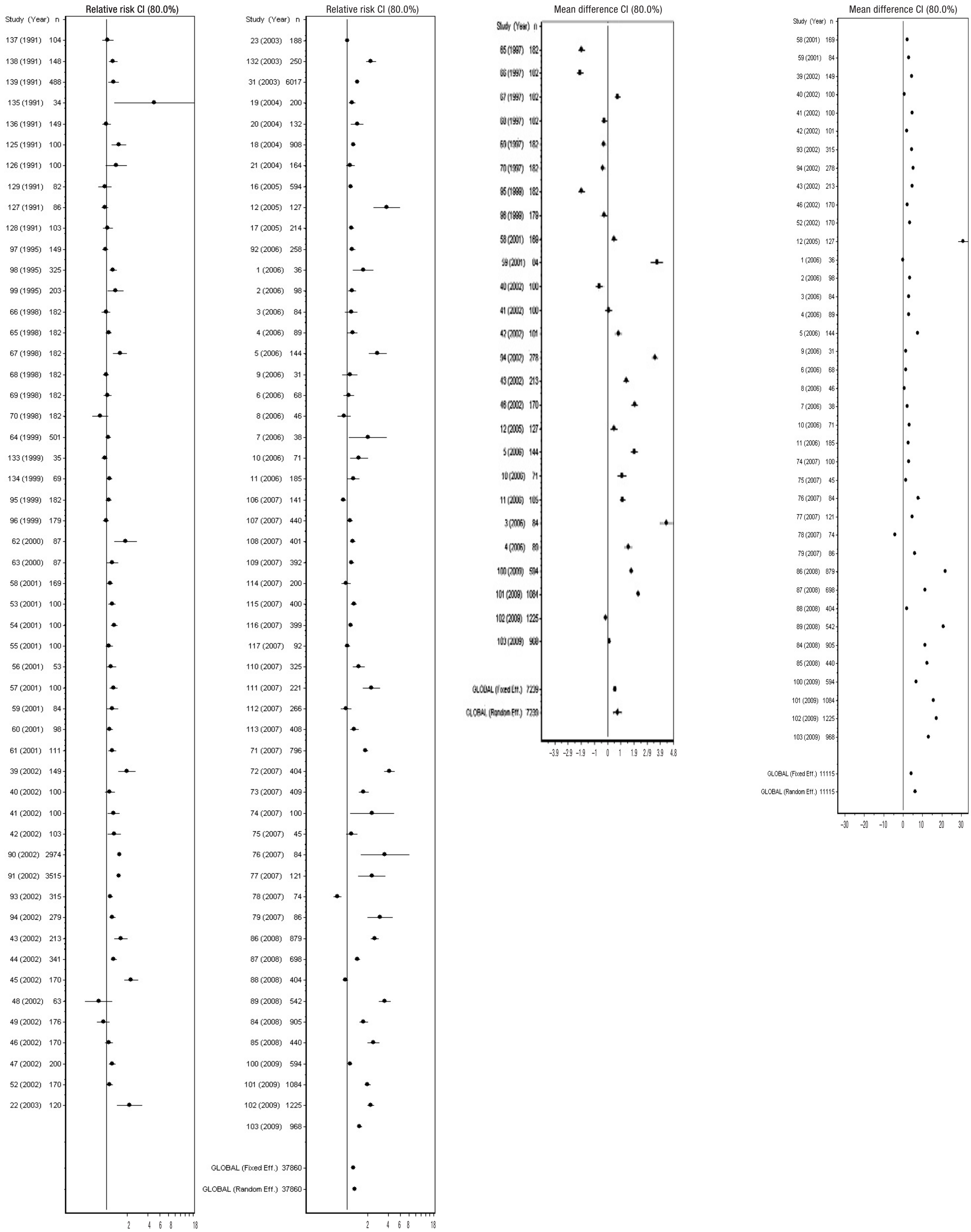

Figure 1. Forest-plots of the meta-analysis on the effect of melatonin implants on the reproductive variables of Spanish farms: RR (relative risk for fertility) (1-2); *d* [standardized mean difference for prolificacy (3) and fecundity (4)]. The numbers correspond to the references in Table 1.

areas separated by a line. On one side are positive values that, in this case, indicate that the treatment increased fertility, prolificacy or fecundity. The negative values indicate that the control group exhibited higher values. The line in the center of Figure 1 corresponds to the value 0, which indicates that the treatments and controls did not differ significantly and, therefore, the null hypothesis of the meta-analysis was not rejected.

Fertility (probability of pregnancy)

The analysis of the effects of melatonin on fertility, a dichotomous variable (pregnancy or not) was based on the results of 105 experiments, which included 37,860 animals. After the construction of 2 × 2 tables (treatment group *vs.* control group and pregnancy *vs.* non pregnancy), the RR or the probability that a ewe is pregnant was calculated. In 16 of the 105 experiments, the RR was < 0, which indicated that sheep in the treatment group had fertility rates that were less than those in the control group. The overall average RR (Table 2) was 1.29, which indicated that the treatment with melatonin increased significantly (by 29%) the likelihood that a ewe became pregnant. In all of the cases analyzed, either in terms of productive aptitude, breed, season or age, melatonin treatments always showed the same trend. The highest RR (1.47) based on the productive aptitude of farms was encountered on mixed-use farms, and Merino sheep showed the most improvement in fertility after the treatment with melatonin (50%). The effect of the treatment on fertility was 5% greater in the anoestrus season than it was in the reproductive period. The RR of adults and ewe-lambs were 1.30 and 1.25, respectively.

Homogeneity statistics indicated that the studies included in the analysis exhibited a high degree of heterogeneity ($p < 0.0000$) (Table 3), which was reflected in the Galbraith and L'Abblé plots (Fig. 2). In the test for publication bias, the Z-statistic (Begg Test) was 4.2048 ($p < 0.0000$) and the *t*-statistic (Egger Test) was 1.0919 ($p = 0.2774$).

Prolificacy (number of lambs born/lambing)

To assess the effects of melatonin treatments on prolificacy, *D* and *d* and their corresponding CI were calculated (Table 4). The statistics and plots of heterogeneity ($p < 0.0000$ in all cases) are presented in Table 3 and Figure 2. In 41 studies (n = 8,700 sheep), values were obtained that met the inclusion criteria. The overall *D* was 0.08; *i.e.,* the groups treated with melatonin increased their prolificacy by 0.08 lambs/lambing in comparison to the control groups. In 11 experiments, the prolificacy of the control group exceeded that of the treatment group and, on 4 of the farms, the null hypothesis could not be rejected; *i.e.,* prolificacy was similar in both groups. Sheep reared for meat exhibited the least improvement ($d = 0.73$) compared to dairy sheep ($d = 1.19$) and mixed-use sheep ($d = 1.15$). Improvement was greater in the Assaf breed than it was in the Rasa Aragonesa breed. The *d* for prolificacy was much high in the reproductive period than it was in the anoestrus period. Melatonin implants had a stronger effect on prolificacy in ewe-lambs ($d = 0.98$) than in adults ($d = 0.87$). The

Table 2. Relative risk (RR) and confidence intervals (CI) obtained after the meta-analysis of 105 experiments performed on Spanish sheep farms to analyze the effect of melatonin implants on fertility (pregnancy or not)

	Studies	n	RR	CI (80%)
Overall	105	37,860	1.2938	1.2531-1.3359
Meat	65	24,818	1.2658	1.2204-1.3130
Dairy	24	3,447	1.2343	1.1531-1.3212
Mixed	16	9,595	1.4749	1.3494-1.6121
Rasa	32	6,141	1.1348	1.0969-1.1740
Assaf	21	9,131	1.3084	1.2044-1.4214
Merino	29	12,890	1.5056	1.3967-1.6230
Anoestrus[1]	84	34,928	1.3022	1.2576-1.3483
Breeding season	21	2,932	1.2565	1.1592-1.3619
Adults	86	35,964	1.3001	1.2564-1.3453
Ewe-lambs	19	1,896	1.2511	1.1436-1.3688

[1] Considering mating from March to July.

Table 3. Dersimonian heterogeneity test from the meta-analysis of the effect of melatonin implants on the fertility (pregnancy or not), prolificacy (number of lambs born/lambing) and fecundity (number of lambs born/100 ewes) of sheep in Spain

	Fertility			Prolificacy			Fecundity		
	Statistic Q (Ji-squared)	d.f.	*p*-value	Statistic Q (Ji-squared)	d.f.	*p*-value	Statistic Q (Ji-squared)	d.f.	*p*-value
Overall	822.4292	104	< 0.001	2,471.8605	40	0.0000	8,939.9462	38	0.0000
Meat	450.1784	64	0.0000	2,011.2614	23	0.0000	7,322.9815	21	0.0000
Dairy	88.6403	23	0.0000	355.2540	12	0.0000	727.9838	12	0.0000
Mixed	142.3599	15	0.0000	30.2340	3	0.0000	62.8807	3	0.0000
Rasa	63.3445	31	0.0005	1,331.7954	17	0.0000	378.9417	9	0.0000
Assaf	120.5320	20	0.0000	354.4582	11	0.0000	720.5714	11	0.0000
Merino	398.7197	28	0.0000	628.5832	4	0.0000	5,988.2773	10	0.0000
Anoestrus	710.3887	83	0.000	1,947.9011	25	0.0000	7,783.2464	23	0.0000
Breeding season	96.8173	20	0.0000	408.2983	14	0.0000	665.1914	14	0.0000
Adults	760.3091	85	0.0000	2,153.1907	25	0.0000	7,782.8011	23	0.0000
Ewe-Lambs	53.6626	18	0.0000	317.7995	14	0.0000	515.2623	14	0.0000

bias tests were negative, with non-significant *p*-values ($Z = 1.4938$, $p = 0.1352$; $t = 0.9162$, $p = 0.3652$).

Fecundity (number of lambs/100 ewes)

The meta-analysis of the effects of melatonin treatments on fecundity was based on 11,115 animals in 39 experiments. The melatonin implants resulted in more than 25 extra lambs born/100 treated ewes. Based on the values of the standardized difference between means (*d*), the greatest effect of melatonin on fecundity (> 28 extra lambs/100 ewes) was achieved on meat sheep farms ($d = 7.98$) and, especially in Merino sheep ($d = 12.27$, 39 extra lambs/100 ewes), and in the anoestrus period ($d = 8.56$, 7.27 extra lambs/100 ewes) (Table 4). The effect of melatonin treatments on fecundity was significantly greater in adults ($d = 8.7$) than in lambs ($d = 2.5$). Like the other characters, the tests revealed high heterogeneity among the studies in the analysis ($p < 0.0000$; Table 3; Fig. 2). In the meta-analysis of the effect of melatonin treatment on fecundity, the probability of publication bias was significant ($Z = 2.8065$, $p < 0.005$; $t = 3.1102$, $p = 0.0036$).

Table 4. Mean raw difference (*D*, number of lambs born/lambing) and standardized mean difference (*d*) obtained from the meta-analysis of 41 experiments performed on Spanish sheep farms to measure the effect of melatonin implants on prolificacy (number of lambs born/lambing) and fecundity (number of lambs born/100 ewes)

	Prolificacy					Fecundity				
	Studies	n	*D*	*d*	CI[1] (80%)	Studies	n	*D*	*d*	CI[1] (80%)
Overall	41	8,700	0.08	0.9089	0.6401-1.1777	39	11,115	25.24	6.3188	5.4069-7.2308
Meat	24	7,054	0.06	0.7283	0.3732-1.0835	22	9,469	28.68	7.9796	6.5992-9.3600
Dairy	13	1,154	0.11	1.1905	0.6631-1.7178	13	1,154	13.78	4.1052	3.1226-5.0877
Mixed	4	492	0.11	1.1458	0.7028-1.5888	4	492	33.24	3.1040	2.2471-3.9609
Rasa	18	2,983	0.04	0.6317	0.1527-1.1108	10	1,530	19.49	3.8986	3.1724-4.6249
Assaf	12	969	0.10	1.1997	0.5877-1.8117	12	969	14.73	4.3235	3.1881-5.4589
Merino	5	4,040	0.10	0.8979	0.2358-1.5600	11	7,908	39.10	12.2725	9.7229-14.8220
Anoestrus[2]	26	7,239	0.07	0.7397	0.4110-1.0683	24	9,654	27.70	8.5611	7.2520-9.8703
Breed. Season	15	1,461	0.11	1.2103	0.7420-1.6786	15	1,461	18.12	2.7820	2.0207-3.5434
Adults	26	7,448	0.07	0.8710	0.5273-1.2148	24	9,863	25.04	8.7417	7.4446-10.0388
Ewe-lambs	15	1,525	0.09	0.9759	0.5585-1.3933	15	1,252	21.81	2.4779	1.8198-3.1361

[1] CI corresponds to *d* values. [2] Considering mating from March to July.

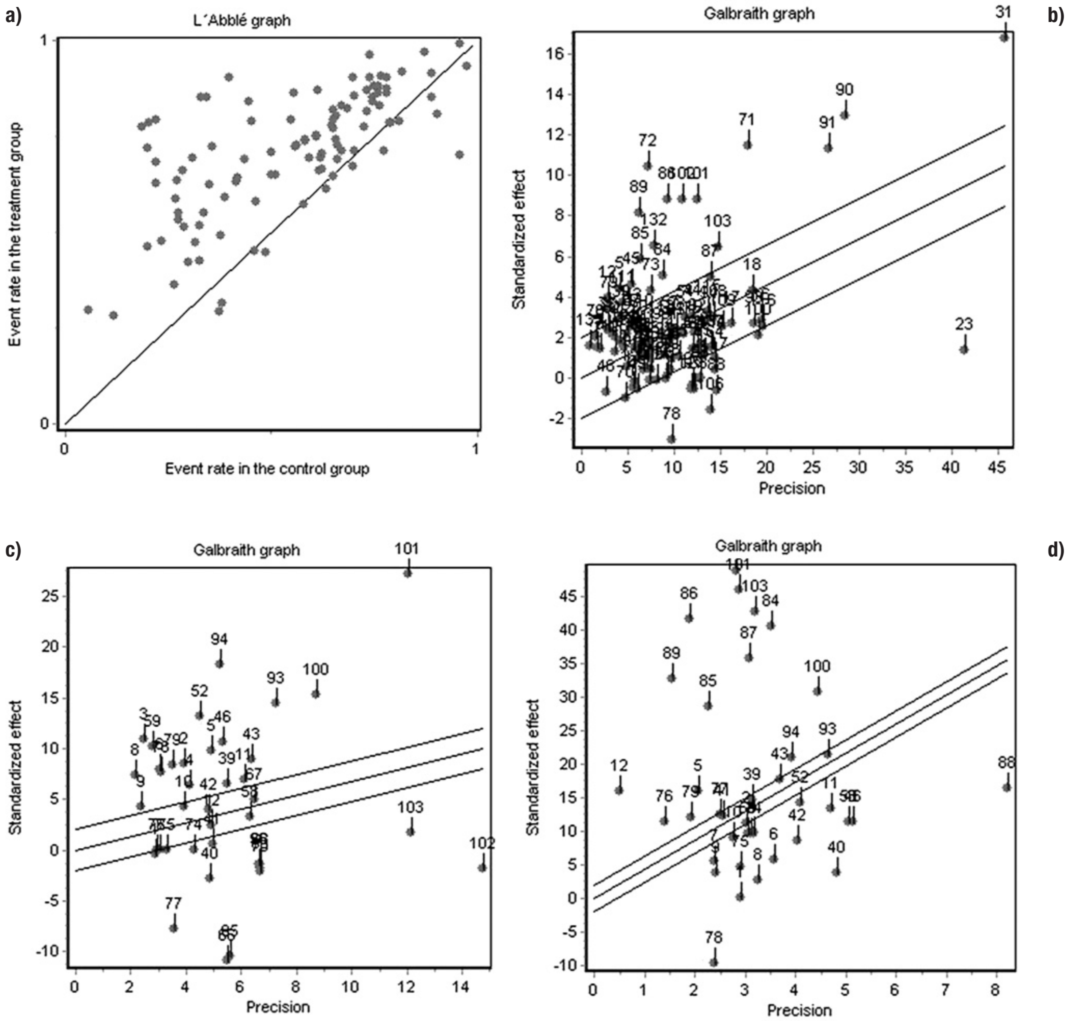

Figure 2. L'Abblé (a) and Galbraith plots derived from the meta-analysis of 105 experiments performed on Spanish sheep farms to measure the effect of melatonin implants on fertility (pregnancy or not, 105 experiments) (b), prolificacy (number of lambs born/lambing, 41 experiments) (c) and fecundity (number of lambs born/100 ewes, 41 experiments) (d). Lines represent confidence intervals. The numbers correspond to the references in Table 1.

Overall results

Table 5 provides a summary of results obtained to provide an overall view of the effect of melatonin implants on sheep reproduction.

Discussion

The main limitation of traditional reviews is the subjectivity of the reviewer in determining which studies are included and the relative importance that s/he places on the results. After a fairly exhaustive search for studies on the subject of interest, each reviewer usually provides his/her particular overall view or summary of the subject, most likely influenced by those authors and studies that seem to be the most relevant or close to their own theoretical position. Consequently, the result of a traditional review will be biased by the experience and in sights of the researcher, his/her personal opinions, preferences, preconceived beliefs, and the personal style with which s/he addresses the review. In contrast, the two main methodological problems of meta-analysis are the possibility of heterogeneity among the studies included in the analysis and so-called publication bias. In the case of the use of melatonin implants in sheep farms, analyses have shown that there can be highly significant heterogeneity and

Table 5. Summary of the results obtained from the meta-analysis of 124 experiments performed on Spanish sheep farms in an 18-yr period to analyze the effect of melatonin implants on fertility (increase in the probability to become pregnant in the groups treated with melatonin), prolificacy (extra lambs born/lambing in the treated ewes), and fecundity (extra lambs born/100 treated ewes)

	Fertility	Prolificacy	Fecundity
Overall	+ 29%	+ 0.08	+ 25.24
Meat	+ 27%	+ 0.06	+ 28.68
Dairy	+ 23%	+ 0.11	+ 13.78
Mixed	+ 47%	+ 0.11	+ 33.24
Rasa	+ 13%	+ 0.04	+ 19.49
Assaf	+ 31%	+ 0.10	+ 14.73
Merino	+ 51%	+ 0.10	+ 39.10
Anoestrus[1]	+ 30%	+ 0.07	+ 27.70
Breed. Season	+ 26%	+ 0.11	+ 18.12
Adults	+ 30%	+ 0.07	+ 25.04
Ewe-lambs	+ 25%	+ 0.09	+ 21.81

[1] Considering mating from March to July.

bias. In theory, the accumulation of data from multiple experiments should enhance the precision and accuracy of the results; however, combining data assumes that the differences among studies are due to chance. In reality, there are likely to be other factors, such as latitude, farm, reproductive management, breed, and age, which contribute to the differences among studies. Therefore, a meta-analysis can produce ambiguous results because the heterogeneity among studies is ignored and, inadvertently, it removes the bias of an experiment merely to compensate (Marín-Martínez *et al.*, 2009). One aspect of heterogeneity, which is usually the clinical or biological differences between studies and differences in procedures, did not apply in our study because the experimental design is almost unique and, usually the study has been supervised by our research group.

Over-interpretation of the results can be induced by attempting to investigate heterogeneity because such investigations are usually inspired, at least to some extent, by looking at the results in hand (Thompson, 1994). Moreover, apparent (even statistically significant) heterogeneity can be due to chance, and searching for its causes would then be misleading. The problem is similar to that of subgroup analyses within an individual clinical trial (Yusuf *et al.*, 1991); however, the degree of heterogeneity among clinical trials is greater than that within individual trials, which is a more serious problem. Guidelines for deciding whether to accept results that stem from an investigation of heterogeneity depend on, for example, the magnitude and statistical significance of the differences identified, the extent to which the potential sources of heterogeneity were specified in advance, and indirect evidence and biological considerations that support the investigation (Stewart and Parmar, 1993). For instance, the high degree of heterogeneity detected in the two meta-analyses published on the use of melatonin to treat sleep disorders in humans (Brzezinski *et al.*, 2005; Buscemi *et al.*, 2006), has not diminished the clinical interest insights revealed by the meta-analysis.

A possible alternative for investigating heterogeneity is to conduct analyses of subgroups. The analysis of subgroups (stratification) involves the meta-analysis of studies within different groups that are defined by common characteristics (*e.g.,* productive aptitude, breed, season, age). In all of the stratifications examined in our meta-analysis, melatonin implants had a positive effect on sheep reproduction. Of note is the effect of melatonin on fertility in the reproductive period (RR = 1.26, CI = 1.2531-1.3359) because, generally, this hormone is used in spring, only, but appears to be beneficial throughout the year. Moreover, the analysis of prolificacy indicated that *d* in the reproductive period was higher than that observed in the anoestrus period, probably because almost all of the studies that included data from this period were on dairy farms, which in turn, proved more effective for this character after the use of melatonin implants.

Selection bias can arise when only a portion of the experiments performed is included in the meta-analysis because the search for studies has not been exhaustive or having tendentiously limited. Although an exhaustive search might identify all of the relevant studies, bias might still arise when the criteria for inclusion in a meta-analysis are defined. If, as is usual, the criteria for inclusion are defined by a researcher familiar with the subject under review, the criteria can be influenced by his/her knowledge of the results in the set of potential studies. Manipulation of the criteria for inclusion might lead to the selective inclusion of studies that have positive findings and the exclusion of studies that have negative findings (Egger and Smith, 1998). In addition, some experiments are not published; particularly those that produced results that were not spectacular or contradicted the prevailing view (Thornton and Lee, 2000). Both the authors who choose not to submit the results for publication and the editors who often reject them are responsible for the resulting publication bias. It is well known that many experiments do not get published and this is most common when the result of the experiment is "negative"; *i.e.,* when significant differences between groups are not demonstrated or when the results are unfavorable to a sponsor's new drug. In those cases, the researcher and the sponsor (typically, a pharmaceutical company) tend to have less interest in submitting an article for publication. Among the studies included in our analysis, 16 reported that the implants had a negative effect on fertility, 15 reported a negative effect on prolificacy, and 1 reported a negative effect on fecundity, so the bias detected in our study should not be attributed to this fact.

In addition, there is evidence that clinical trials in which there are no significant differences detected take longer to be published (Egger and Smith, 1995). For those reasons, a meta-analysis that only includes published studies tends to give a biased result. Furthermore, some experiments are published more than once, but in seemingly different forms; the duplicated study can introduce a bias that favors the trend of the results of the experiments that have been subject to duplicate publication (Tramer *et al.*, 1997). In a portion of meta-analyses, clinical trials published in languages other than English were ignored and the proportion of experiments that reported "negative" findings is highest among those published in non-English languages (Gregoire *et al.*, 1995). Given its nature, it is very difficult to prevent publication bias or, at least, estimate the magnitude of the problem.

In conclusion, the meta-analysis reported here does lend statistical support to the proposition that melatonin implants can improve lamb production in a wide variety of breeds, farms, seasons, and ages. Overall, the results revealed a 29% improvement in fertility, 0.08 lambs more per lambing, and 0.25 extra lambs per ewe treated with melatonin implants. The meta-analysis to evaluate the effectiveness of melatonin implants in sheep to increase the production of lambs revealed a significant effect of this hormone on the likelihood that a ewe became pregnant, increased the number of lambs born per lambing, which ultimately increased the number of lambs born per treated ewe. This not only confirmed what has been reviewed previously in the descriptive literature, but by means of statistical procedures, synthesized and more accurately estimated the effect of this hormone on sheep.

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