

Effect of melatonin on  
deseasonalization and reproduction  
in small ruminants: last update

**השפעת המלטונין**

**להפחתת העונתיות ברביית צאן: עדכון**

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de Aragón**

**Universidad Zaragoza**

# SEASONAL SEXUAL ACTIVITY OF SMALL RUMINANTS

## עונתיות הפעילות המינית בצאן

- SHEEP: SEASONAL POLIESTRIC SPECIES

- כבשים: מספר מחזורי ייחום בעונה

- Seasonal anestrus - תקופות עונתיות של חוסר פעילות מינית

- Breeding season - עונת רבייה



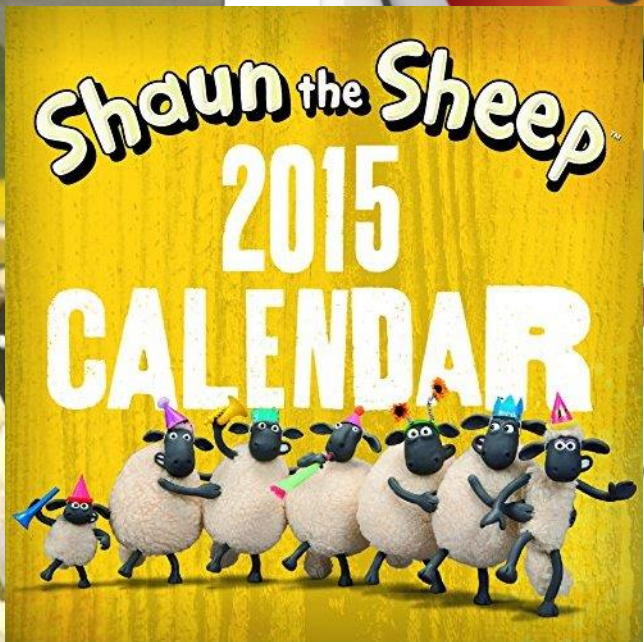
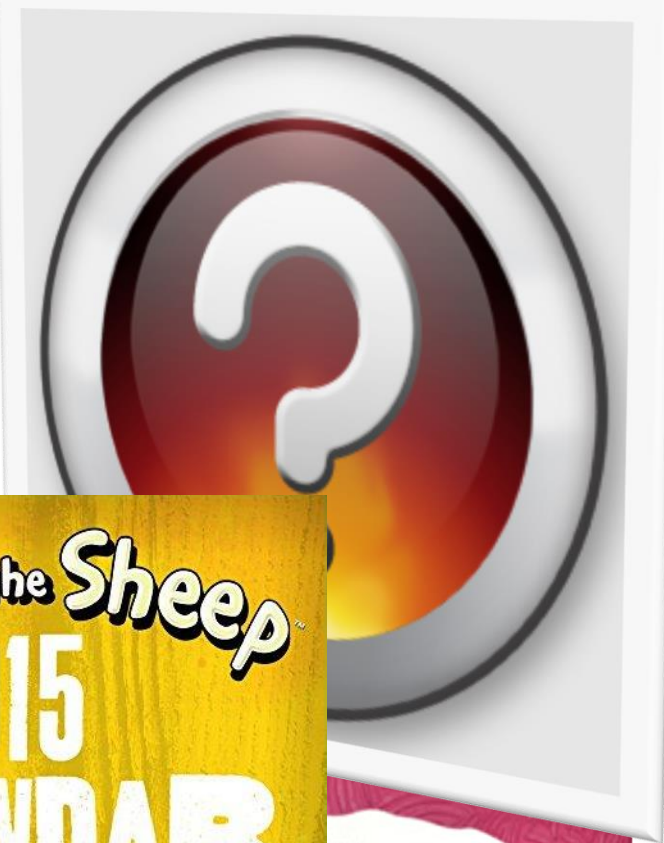
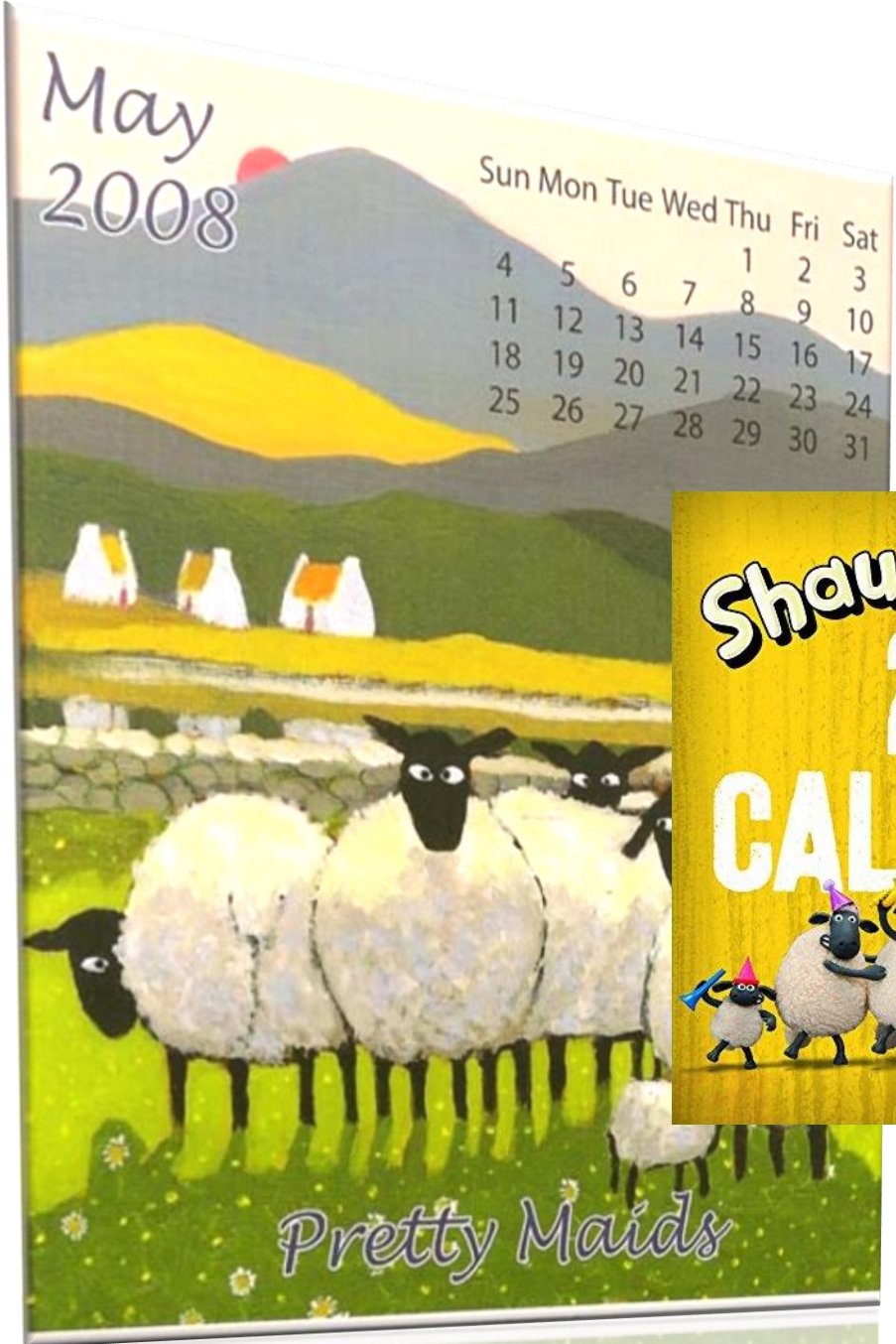
- WHY?? מדוע??

- Pregnancy 5 months

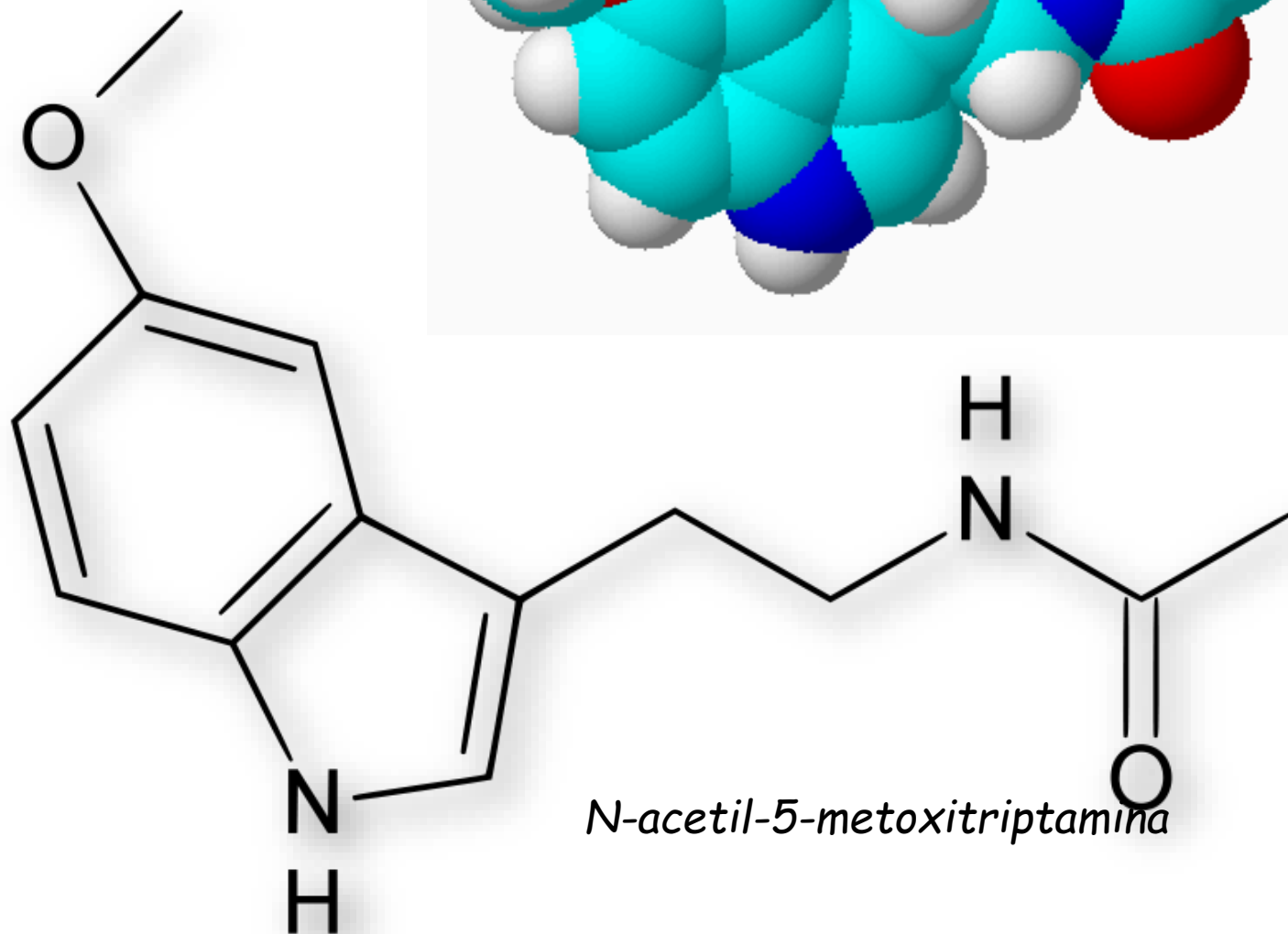
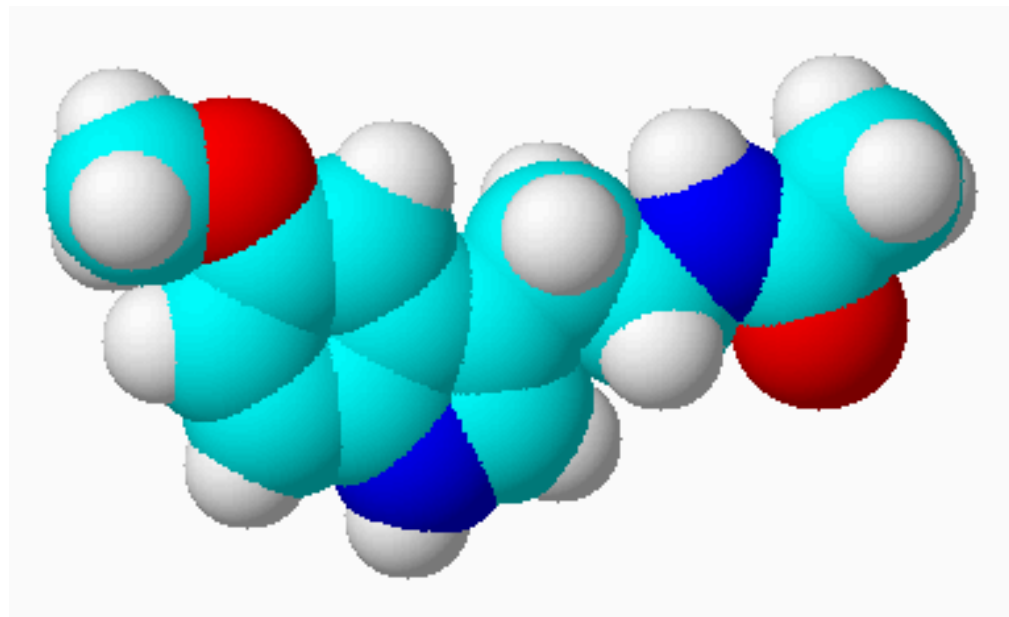
- משך הריון 5 חודשים

- Lambing during optimus herbage supply

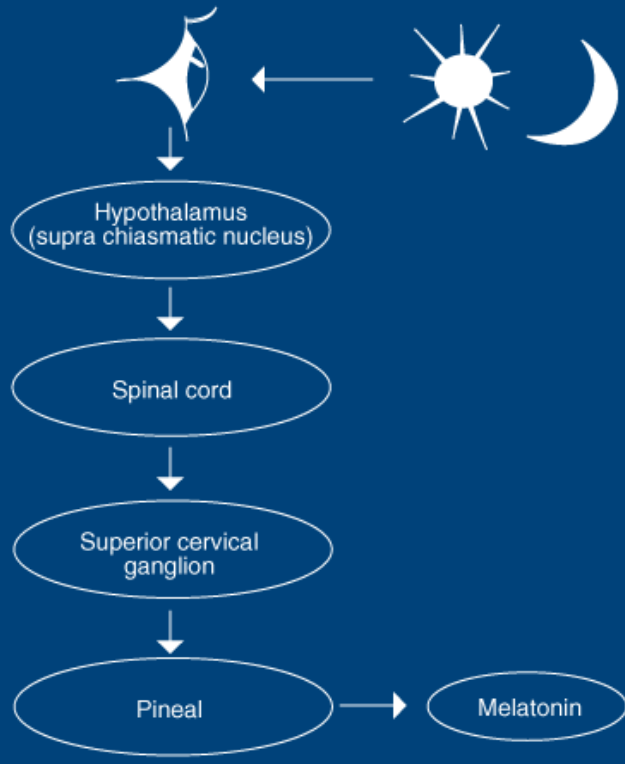
- המלטה במהלך התקופה האופטימלית של העשב במרעה



מולקולת מלטונין



*N-acetil-5-metoxitriptamina*

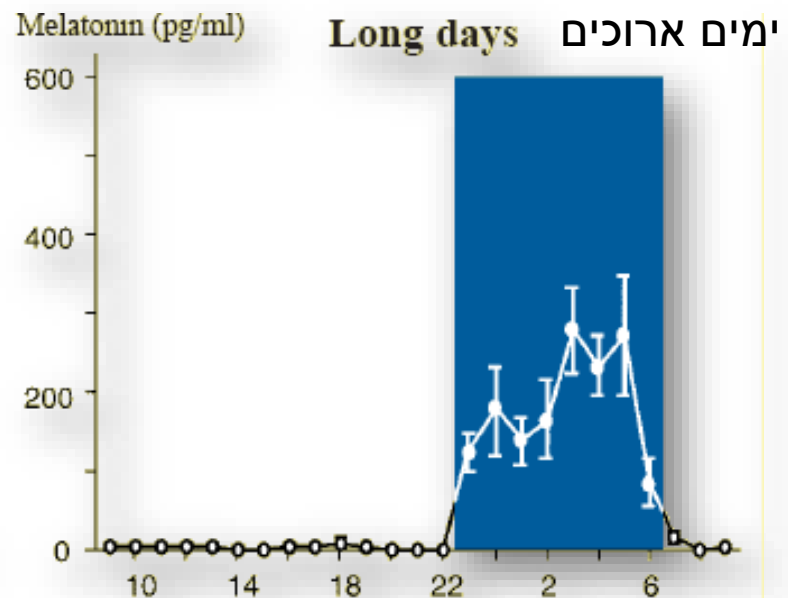
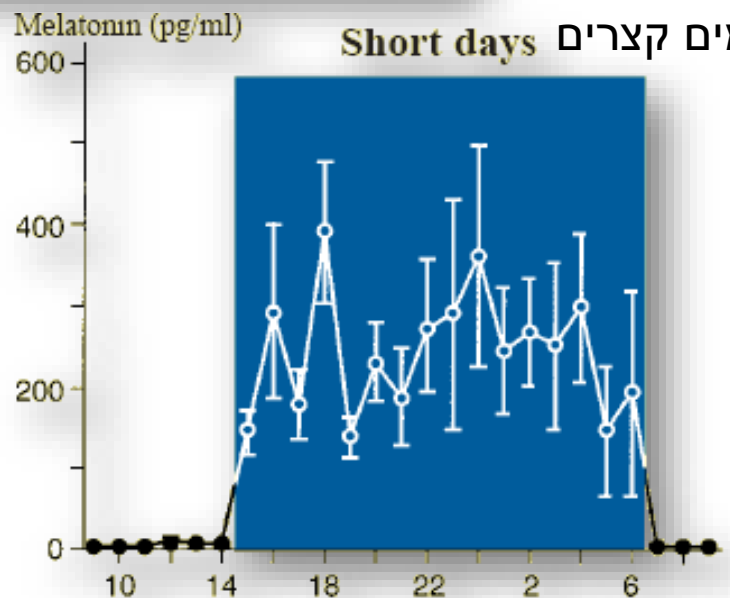


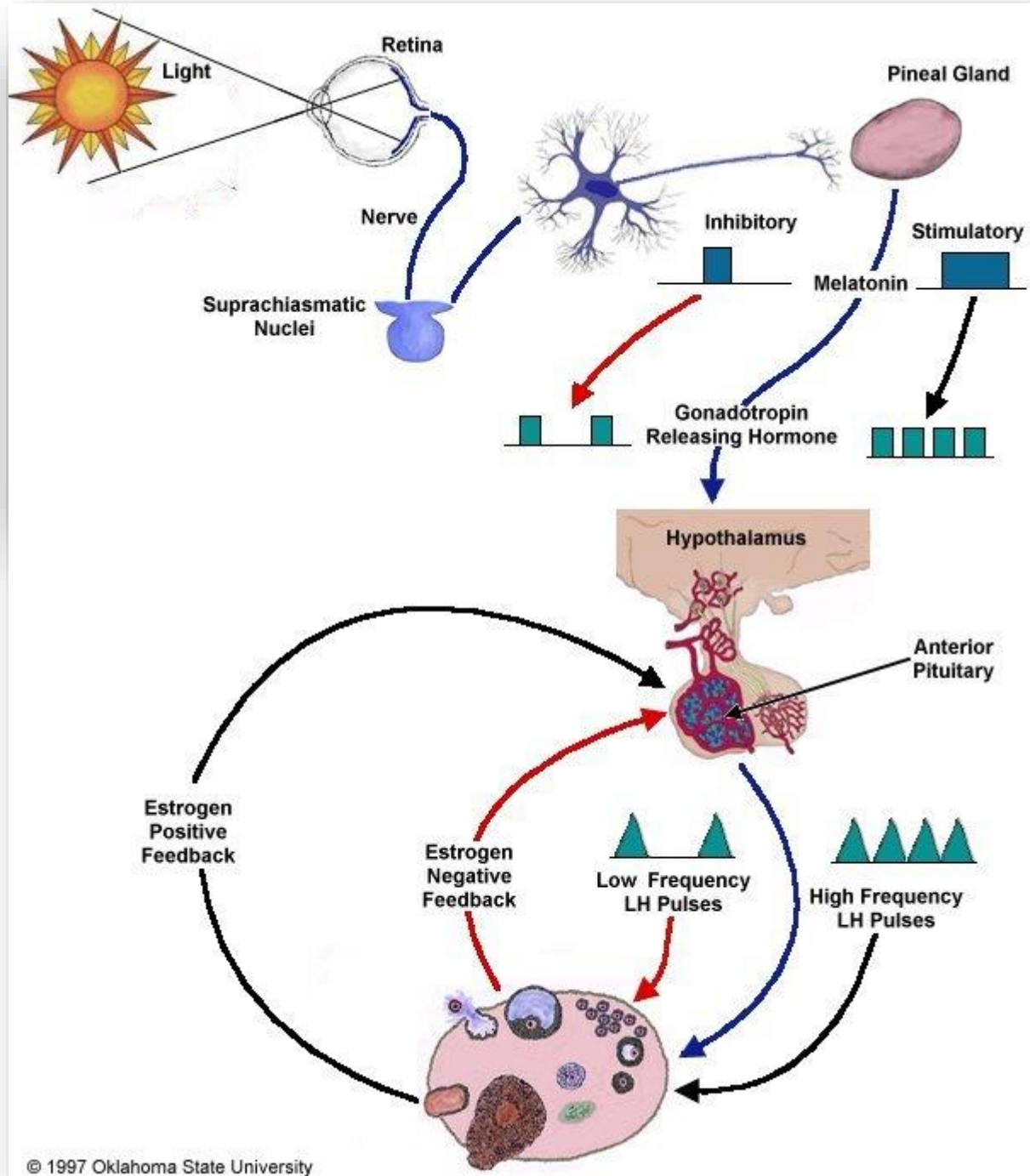
## Seasonal Breeding Cycle in Sheep

עונתיות מחזורי רבייה בכבשים



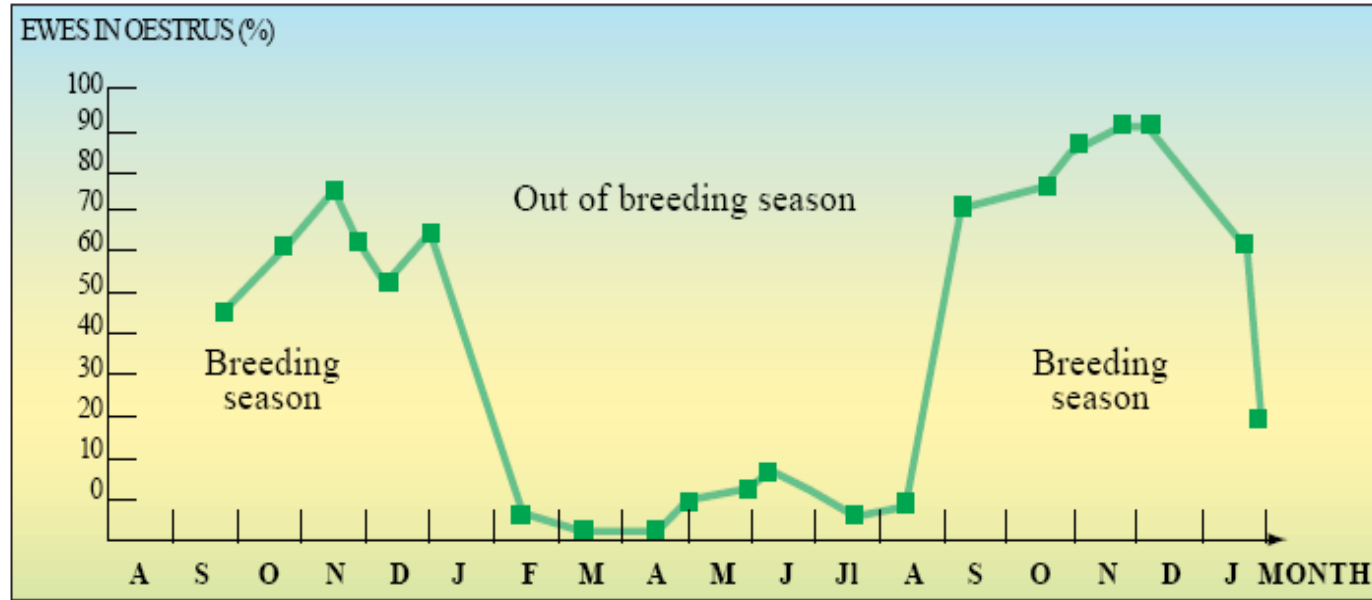
Scient. 1999





Seasonal changes  
in reproduction  
in Ile de France sheep  
according to THIMONIER  
and MAULEON - 1969

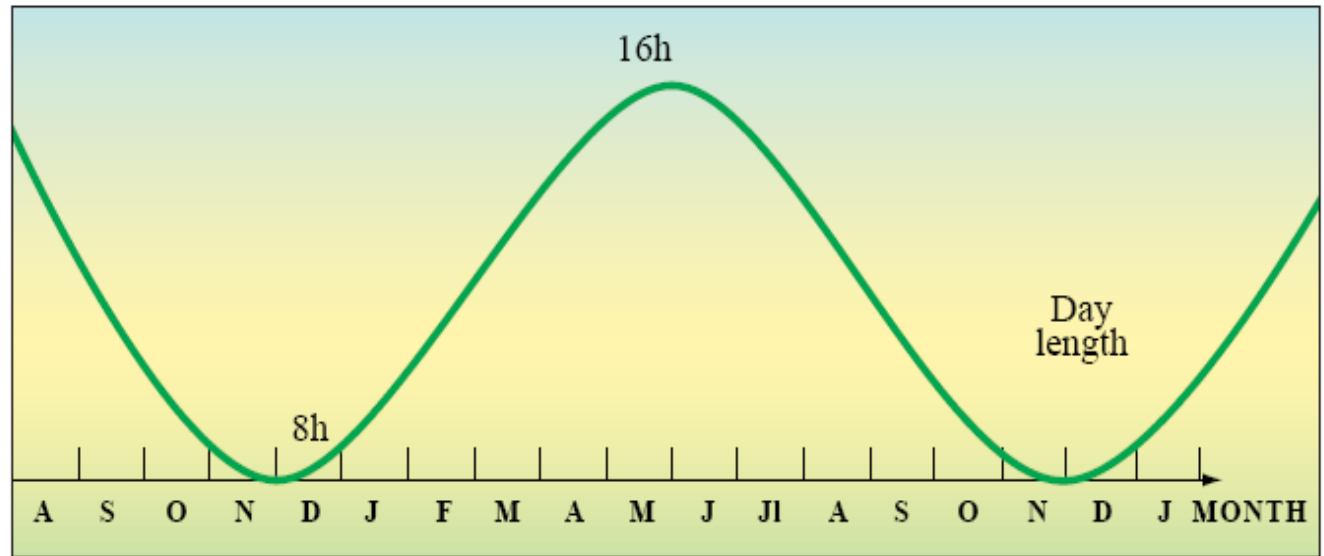
עונתיות הרבייה  
בכבשי איל דה פרנס



(Fig. 8)

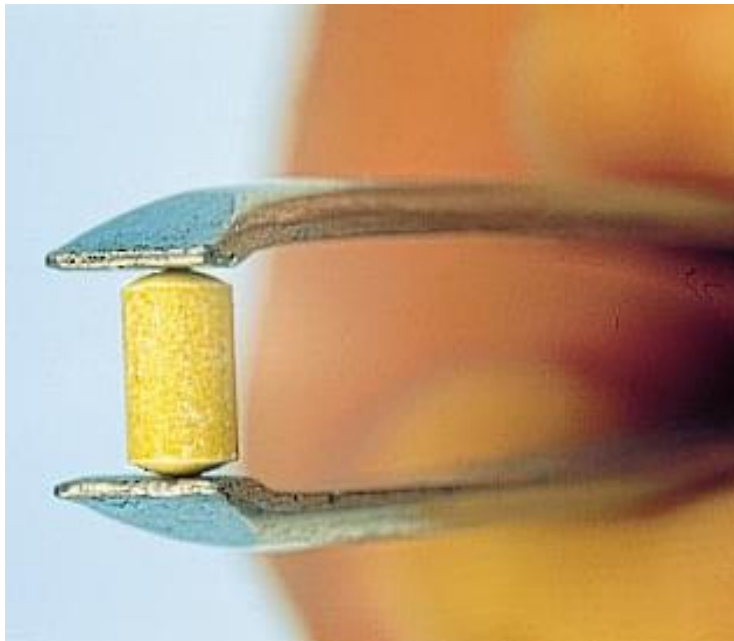
Variation in number  
of daylight hours in the year  
(at a latitude corresponding to France)

שונות שעות אור יום  
לאורך השנה בצרפת



# MELATONIN IMPLANTS

## שתלי מלטונין





# Melatonin    מלטונין

החדרת השתל  
הוצאת איילים

IMPLANTS  
RAMS OUT

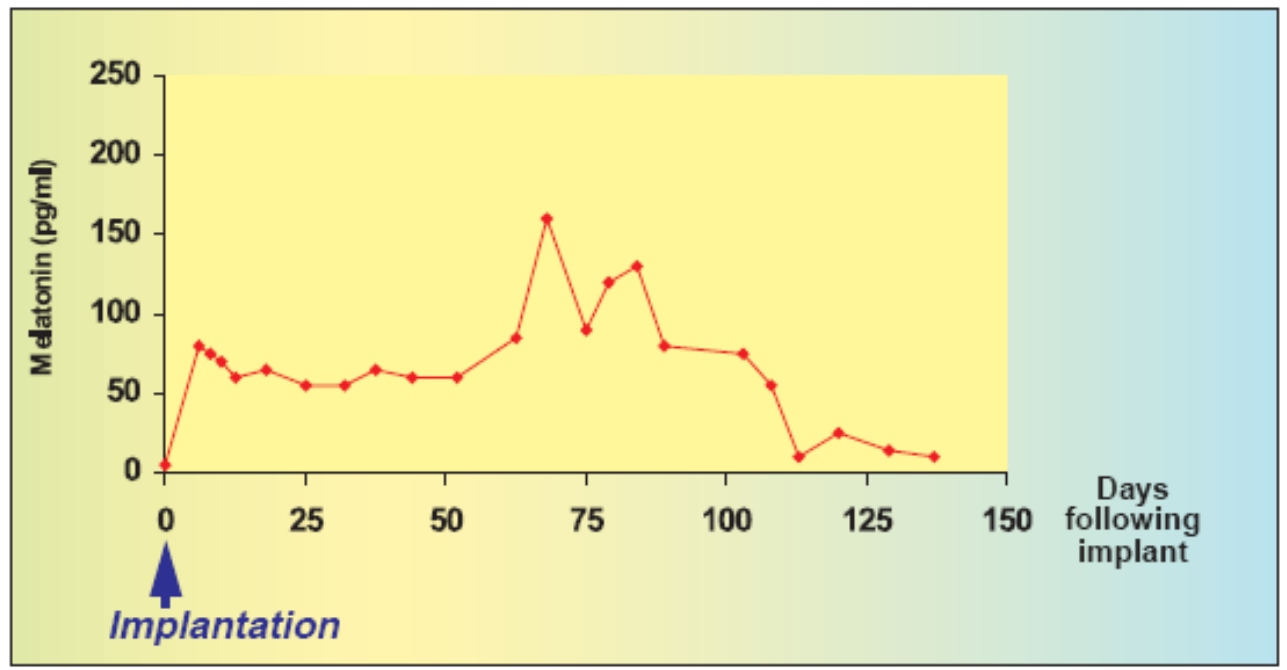
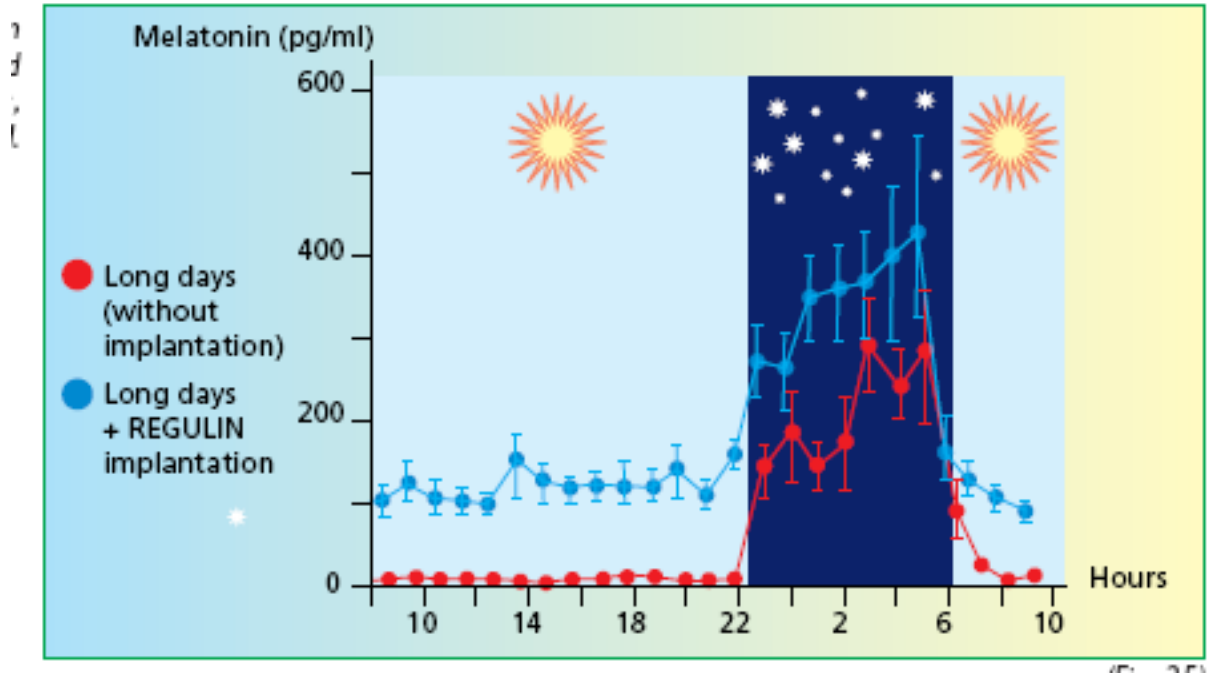
35-42 días

החזרת האיילים  
לכבשים - הרבעה

Introducción de machos  
Inicio de cubrición

RAMS IN





# מטא-אנליזה ליעילות שתל מלטונין לשיפור ביצועי הרבייה בכבשים

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)  
Available online at [www.inia.es/sjar](http://www.inia.es/sjar)

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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.

# סיכום תוצאות 124 ניסויים במשקי צאן בספרד לבחינת השפעת מלטונין על פוריות

**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 aptitud and breed are indicated

Reference	Aptitud, breed	Reference	Aptitud, breed	Reference	Aptitud, breed
1. Felch et al., 1991a	Meat RA	43. Forcada et al., 2002	Meat RA	85. Palacios et al., 2006	Meat RA
2. Felch et al., 1991a	Meat RA	44. García et al., 2002	Mixed XxLA	86. Palacios et al., 2006	Meat RA
3. Felch et al., 1991a	Meat RA	45. Mantu et al., 2002a	Meat ME	87. Palacios et al., 2006	Meat RA
4. Felch et al., 1991b	Meat RA	46. Mantu et al., 2002b	Meat RIxCA	88. Palacios et al., 2006	Meat LA
5. Felch et al., 1991b	Meat MA	47. Mantu et al., 2002b	Meat RIxCA	89. Palacios et al., 2006	Dairy AS
6. López and Balcázar, 1991	Meat RA	48. Mejías et al., 2002	Meat MExSE	90. Palacios et al., 2006	Dairy AS
7. López and Balcázar, 1991	Meat RA	49. Palacios et al., 2002	Dairy AS	91. Palacios et al., 2006	Mixed CH
8. López and Balcázar, 1991	Meat T	50. Palacios et al., 2002	Dairy AS	92. Palacios et al., 2006	Mixed MA
9. López and Balcázar, 1991	Mixed CH	51. Palacios et al., 2002	Mixed CH	93. Toppojo et al., 2006	Dairy LA
10. López and Balcázar, 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. Valera, et al., 2002	Meat RA	95. Abecia et al., 2007	Meat RA
12. Gómez et al., 1995	Meat ME	54. García et al., 2003	Mixed XxLA	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. Mantu, 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. Felch and Akhtar, 1999	Meat RA	63. Palacios et al., 2003	Dairy AS	105. Abecia et al., 2007	Dairy AS
22. Felch and Akhtar, 1999	Meat RA	64. Prieto et al., 2003	Meat MA	106. López and Sánchez, 2007	Mixed ME
23. Forcada et al., 1998	Meat RA	65. Sánchez et al., 2003	Meat MExFL	107. López and Sánchez, 2007	Mixed ME
24. Forcada et al., 1998	Meat RA	66. Sánchez et al., 2003	Meat MExFL	108. López and Sánchez, 2007	Mixed ME
25. Legaz et al., 2000	Dairy MAxAS	67. Sánchez et al., 2003	Meat MExFL	109. Tamayo et al., 2007	Dairy AW
26. Legaz et al., 2000	Dairy MAxAS	68. Sánchez et al., 2003	Meat MExFL	110. Tamayo et al., 2007	Dairy AS
27. García et al., 2001	Meat ME	69. Sánchez et al., 2003	Meat MExFL	111. Tamayo et al., 2007	Dairy ASx CH
28. García et al., 2001	Meat MEx FL	70. Sánchez et al., 2003	Meat MExFL	112. Tamayo et al., 2007	Dairy AW
29. García et al., 2001	Meat MEx CHA	71. Santander et al., 2003	Meat RA	113. Tamayo et al., 2007	Dairy ASx CH
30. García et al., 2001	Meat MEx 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. Arribola et al., 2008	Meat ME
32. Magueta et al., 2004	Meat ME	74. Prieto et al., 2004	Mixed MA	116. Arribola et al., 2008	Meat ME
33. Palacios et al., 2004	Dairy AS	75. Ramirez et al., 2004	Mixed MA	117. Arribola et al., 2008	Meat ME
34. Pizarro et al., 2001	Meat SE	76. Argente et al., 2005	Meat RA	118. Arribola et al., 2008	Meat ME
35. Pizarro et al., 2001	Meat SE	77. Argente et al., 2005	Meat RA	119. Arribola et al., 2008	Meat ME
36. Abecia et al., 2002	Meat RA	78. Argente et al., 2005	Meat RA	120. Arribola et al., 2008	Meat ME
37. Abecia et al., 2002	Meat RA	79. Arribola et al., 2005	Meat ME	121. Arribola et al., 2009	Meat ME
38. Abecia et al., 2002	Meat RA	80. Palacios et al., 2005	Dairy AS	122. Arribola et al., 2009	Meat ME
39. Abecia et al., 2002	Meat RA	81. Roppejo et al., 2005	Dairy AS	123. Arribola et al., 2009	Meat ME
40. Carbonero et al., 2002	Mixed CR	82. Gómez et al., 2006	Dairy MA	124. Arribola 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: Charolais, FL: Fleischschaf, LA: Lacanue, MA: Manchega, ME: Merino, MP: Merino Pireno, RA: Rasa Aragonesa, RI: Ripollina, T: Talavera, X: Xisqueta.

**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 <sup>1</sup>	+ 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

<sup>1</sup> Considering mating from March to July.

Fertility  
Prolificacy  
Fecundity

תוספת בשיעור התעבורת  
תוספת ולדות להמלטה  
תוספת במספר הטלאים ל-100 כבשים מטופלות



**MELATONIN  
IN THE RAM**

**מלטונין לאיל**

# MELATONIN-SEMEN QUALITY

## מלטונין – איכות הזרמה

### MELATONIN מלטונין

#### +++ RAM'S SEXUAL ACTIVITY

#### פעילות מינית של האיל

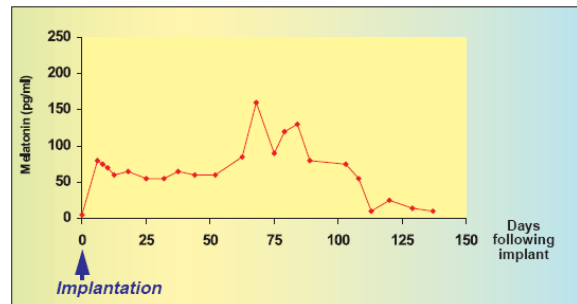
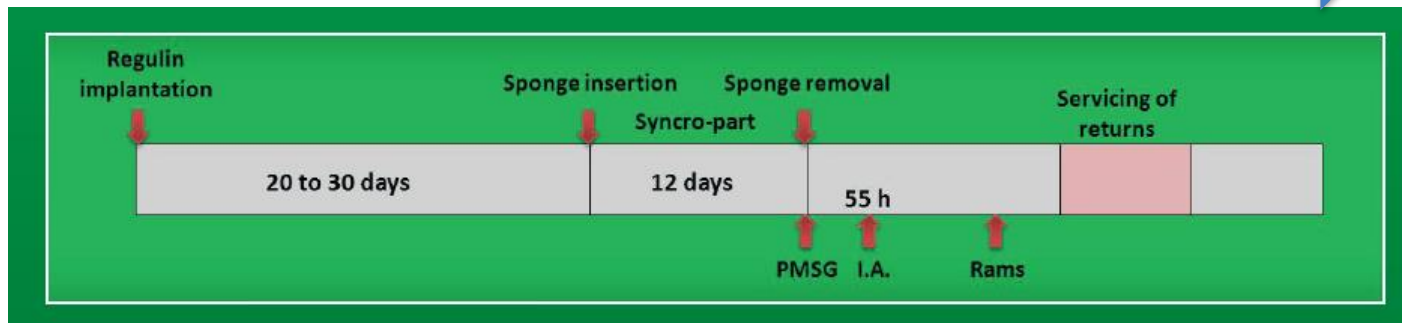
- ++ TESTICULAR TISSUE רקמות האשכים
- ++ NUMBER OF SPERMATOZOA מספר תאי הזרע
- ++ TESTOSTERONE טסטוסטרון
- ++ SOME SEMEN QUALITY PARAMETERS פרמטרים באיכות הזרמה
- ++ NUMBER OF LAMBS BORN FROM EWES MATED WITH TREATED RAMS

מספר הטלאים שנולדו מכבשים שהורבעו ע"י אילים מטופלים

# ARTIFICIAL INSEMINATION: USE OF MELATONIN IN EWES

הזרעה מלאכותית: השימוש במלטונין בכבשים

MELATONIN



## EWE lambs

Comparison of  
SYNCRO-PART with  
REGULIN + SYNCRO-PART  
In one-year-olds ewes  
(CIOP 6.4)

Syncro-Part = sponges

Year	Numbers	SYNCRO-PART		REGULIN + SYNCRO-PART	
		Fertility	Prolificacy	Fertility	Prolificacy
1994	120	70.0%	1.38	75.0%	1.58
1995	48	57.7%	1.40	63.6%	1.80
	120	66.7%	1.34	70.3%	1.39
1996	187	48.9%	1.29	56.8%	1.33
Total	475	59.8%	1.34	65.4%	1.47
Fecundity	475	80.1		96.1	

Regulin with sponges etc = 16 lambs/100 ewes more than sponges only

### MELATONIN IN AI:

**++ PERCENTAGE OF PREGNANT EWES**

**++ NUMBER OF LAMBS BORN/INSEMINATED**

**EWE**

**מלטונין בהזרעה מלאכותית:**

**++ אחוז התעברות הכבשים**

**++ מספר הטלאים שנולדו/כבשים מוזרעות**



# Melatonin on in vivo-embryo production programs

## מלטונין בתכניות יצירת עוברים in vivo

- Donor ewes כבשים תורמות
- Recipient ewes כבשים מקבלות



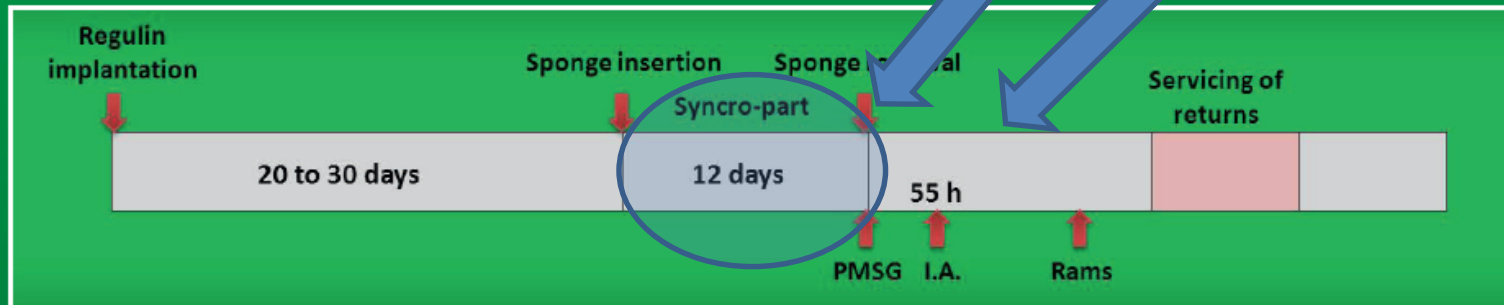
Recommended programs using Regulin with sponges & PMSG are

### Ewes

- At least 30 days after lambing isolate rams (or 7 days before implanting with Regulin)
- 32-42 days before joining implant with Regulin
- Approximately 14 days prior to joining insert sponge
- Remove sponge & treat ewe with PMSG

Embryo collection or embryo transfer

Superovulation treatment



## USE OF REGULIN IN AI & ET PROGRAMMES

### Important Facts:

Regulin will only bring sheep forward by 2 months from their normal breeding time.  
 Sheep have to have enjoyed some long days (summer) before they respond to long nights.  
 Certain rams are lazy out of season and benefit from implanting with 2 Regulin implants a week before the ewes

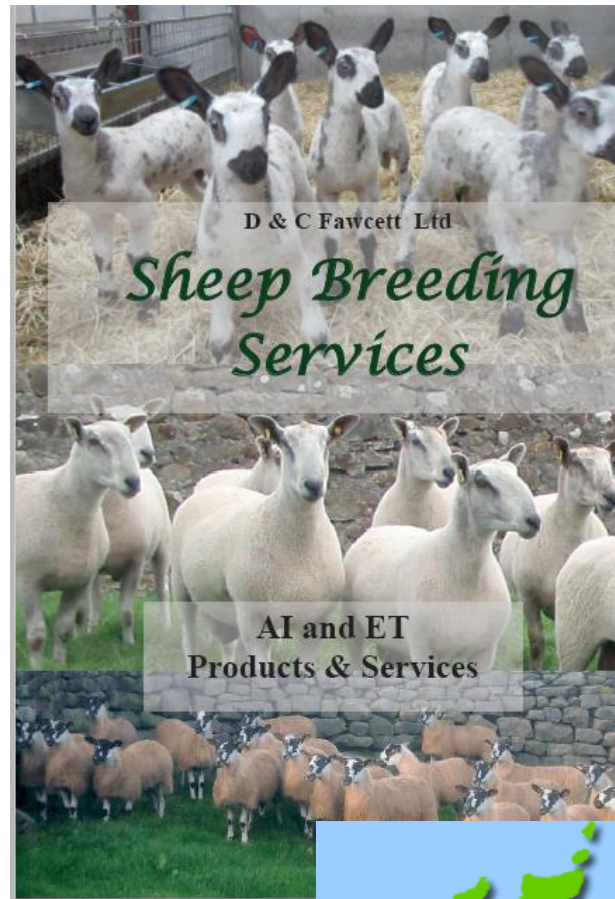
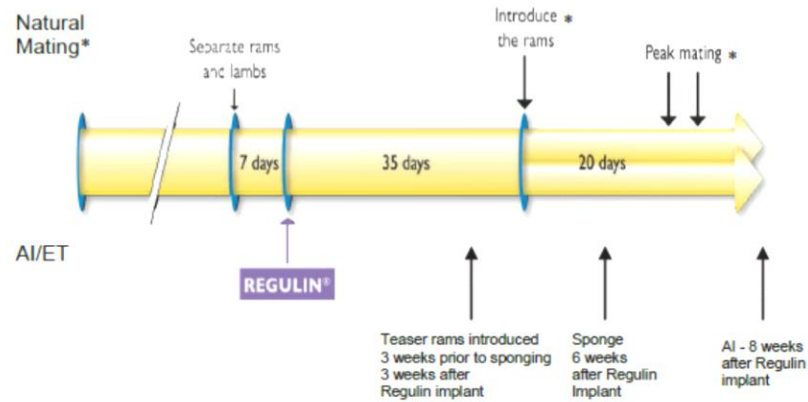
Teasers can be used 10-14 days at day 42 before rams go in at day 52-56 |

The earlier you bring sheep forward the greater delay to peak tupping.

The 35 day gap between implanting ewes and introducing rams can be lengthened or shortened several days (for natural mating).

**GO TO WEBSITE for PICTURES OF HOW TO PLACE REGULIN IMPLANTS**

### EXAMPLES OF THE USE OF REGULIN:



# מלטונין וחיוניות העוברים בכבשים

CSIRO PUBLISHING

*Reproduction, Fertility and Development*, 2019, **31**, 82–92  
<https://doi.org/10.1071/RD18308>

## Role of melatonin on embryo viability in sheep

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# Proposals for MOET and ART protocols including melatonin

- *Forcada et al., 2006*
- Two consecutive superovulations of aged high-genetic value donor ewes
- MELATONIN IMPLANTS
- *Zhang et al., 2013*
- MELATONIN treatment donor and recipient ewes
- Adult vs. Young ewes



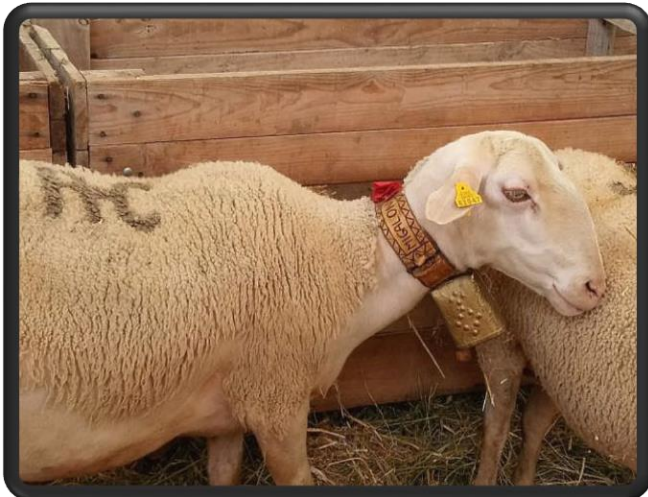
# שימוש במלטונין בפרוטוקולי העברת עוברים

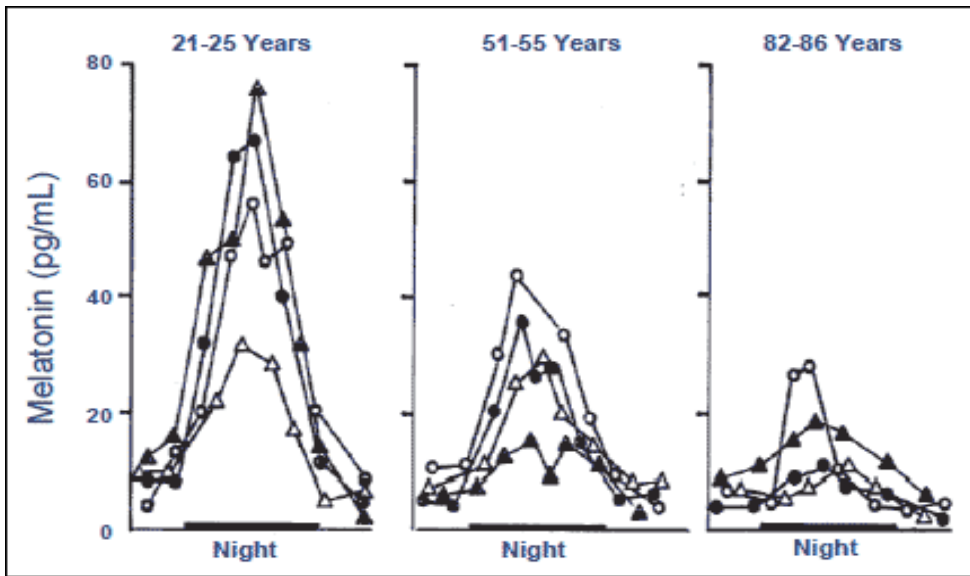
- *Forcada et al., 2006*

- רצף של 2 ביוצים מרובי עוברים של כבשים בוגרות בעלות ערך טיפוחי גבוה שתלי מלטונין

- *Zhang et al., 2013*

- טיפול במלטונין בכבשה תורמת ומקבלת כבשים בוגרות מול צעירות





Effect of ageing  
on melatonin secretion

*Bubenik and Konturek, 2011*

השפעת הגיל  
על הפרשת מלטונין

## OUR PROPOSAL:

The effect of melatonin implants during the seasonal anestrus on embryo production after superovulation in aged high-prolificacy ewes

*Forcada et al., 2006*

ההצעה שלנו:

השפעת שתלי מלטונין מחוץ לעונת הפוריות, על יצירת העובר לאחר ביוץ יתר בכבשים ולדניות בוגרות

> 10 years old-high genetic merit ewes (number of lambs born/lambing)  
כבשים בנות 10 שנים בעלות ערך טיפוחי גבוה לולדנות (מספר טלאים/המלטות)



**+ 30 days: superovulation FSH**



**+ 80 days: superovulation**





	Melatonin-implanted		Control	
	Recovery 1	Recovery 2	Recovery 1	Recovery 2

Ovulation rate	11.9	12.5	12.9	11.1
No. of fertilized embryos	4.2	4.4	4.9	4.1
Fertilization rate (%)	58.6	70.8	51.7	65.4
No. of non-viable embryos	0.6	0.4	0.7	1.3
Rate of non-viable embryos (%)	7.2	4.2	9.3	22.4
No. of blastocysts	2.3	2.4	2.5	1.1
Rate of blastocysts (%)	29.7	36.7	24.1	16.3
No. of viable embryos	3.6	4.0	4.2	2.8
Viability rate (%)	51.3	66	42.3	42.9
No. of freezable embryos <sup>b</sup>	3.1	3.4	3.6	2.2
Freezability rate (%)	43.7	54.5	37.1	33.2

- **Exogenous melatonin can improve the viability of embryos collected from high-prolificacy aged ewes after superovulation in the seasonal anestrous period.**
- **However, the effect of melatonin occurs at medium term, 3 months after implantation, increasing the rates of blastocysts, viability and freezability of embryos because of a decreasing number and rate of non-viable embryos.**





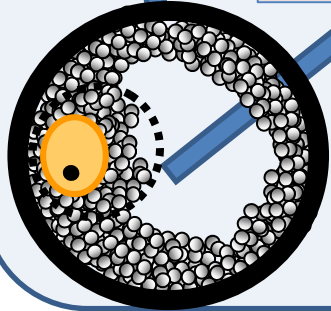
	Melatonin-implanted		Control	
	Recovery 1	Recovery 2	Recovery 1	Recovery 2

Ovulation rate	11.9	12.5	12.9	11.1
No. of fertilized embryos	4.2	4.4	4.9	4.1
Fertilization rate (%)	58.6	70.8	51.7	65.4
No. of non-viable embryos	0.6	0.4	0.7	1.3
Rate of non-viable embryos (%)	7.2	4.2	9.3	22.4
No. of blastocysts	2.3	2.4	2.5	1.1
Rate of blastocysts (%)	29.7	36.7	24.1	16.3
No. of viable embryos	3.6	4.0	4.2	2.8
Viability rate (%)	51.3	66	42.3	42.9
No. of freezable embryos <sup>b</sup>	3.1	3.4	3.6	2.2
Freezability rate (%)	43.7	54.5	37.1	33.2

- **מלטונין ממקור חיצוני, יכול לשפר את חיוניות העוברים הנאספים מכבשים ולדניות, לאחר ביוץ מרובה עוברים מחוץ לעונה.**
- **עם זאת, השפעת המלטונין מתרחשת בטווח בינוני, 3 חודשים לאחר ההשתלה, מגדילה את אחוז הבלסטוציסטים (שלב מוקדם בהתפתחות העוברים), החיוניות ועמידות בהקפאת עוברים, בשל המספר ההולך וקטן של עוברים שאינם חיוניים.**

Granulosa cells: melatonin receptors  
↓p53 gene ↑Bcl-2 and LHR gene

Cumulus cells: synthesize melatonin  
protect DNA damage



1

# MELATONIN מלטונין



10,11

Embryos: ↑quality  
regulation DNA methylation genes

2,3,4,5

IN VITRO: ↓oxidative stress  
↓ DNA damage



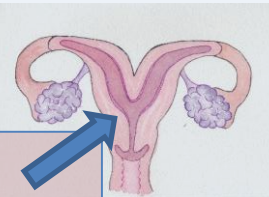
Follicle: antioxidant activity  
↑ oocyte competence



Corpus Luteum: ↑P4 secretion

7,8,9

Uterus: ↓ PGF2α secretion  
↑P4 receptor population



At least 60 days before service, insert three melatonin implants in rams used for natural mating or semen collection for AI to enhance sperm quality

8–10 weeks before embryo collection or embryo transfer, insert one melatonin implant to donor and recipient ewes

העברת עוברים  
לכבשה התורמת והנתרמת

זכר תורם  
הזרמה

RAM

DONOR  
EWE

RECIPIENT  
EWE

MELATONIN

melatonin can be added to the IVM medium, and similar melatonin concentrations can be maintained in the IVF and IVC media until the blastocyst stage is reached

SPERM  
PRESERVATION  
melatonin can also be included in the semen preservation medium

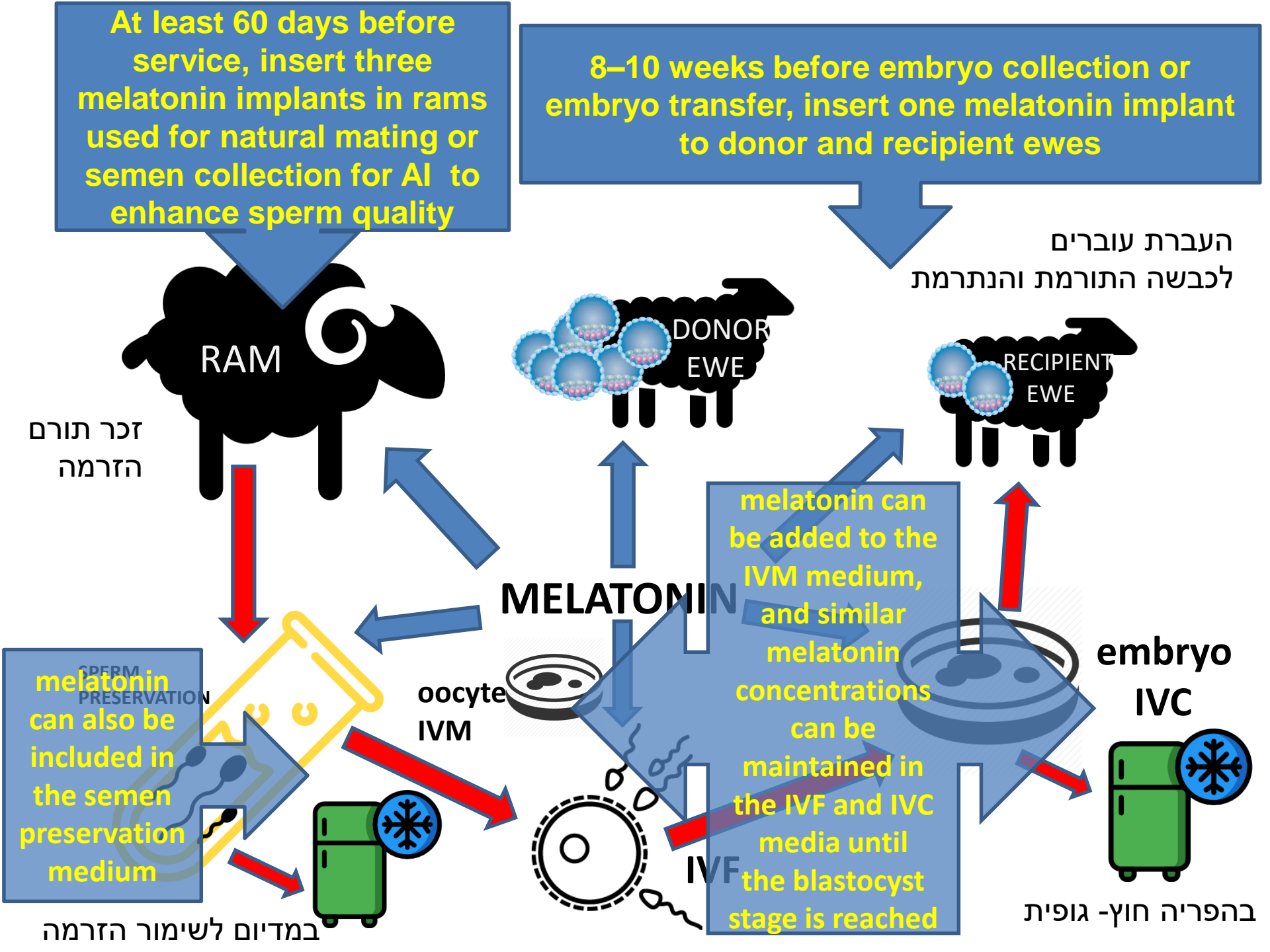
oocyte  
IVM

IVF

embryo  
IVC

במדיום לשימור הזרמה

בהפריה חוץ- גופית



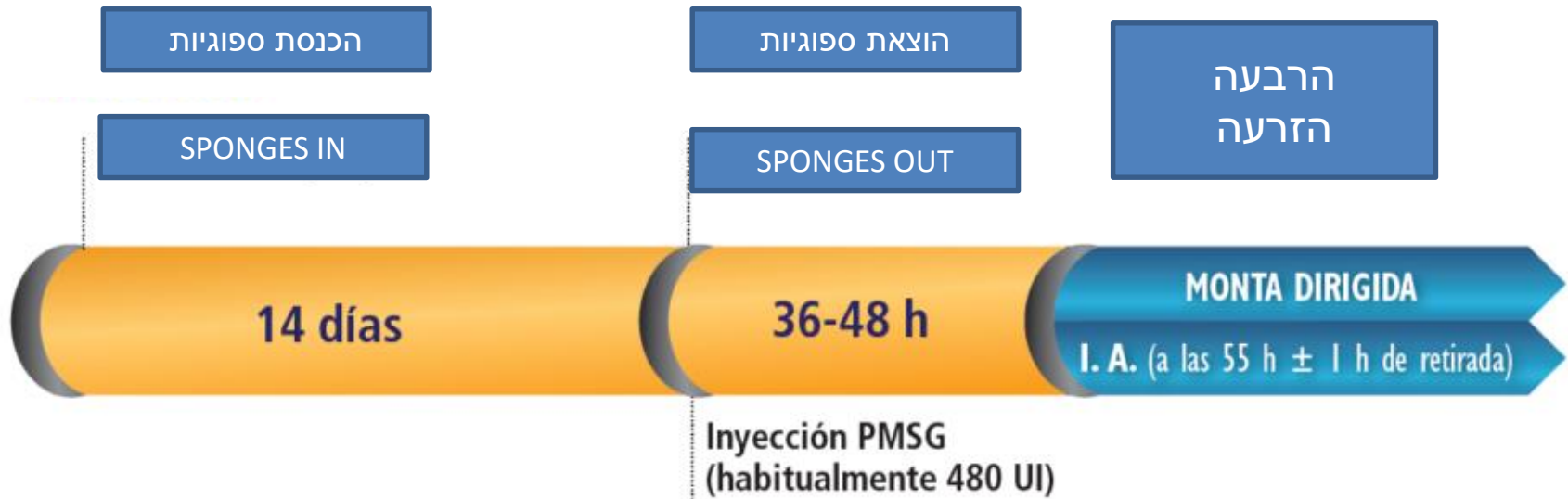
# Protocols to induce oestrus

## פרוטוקולים להשראת ייחום

- **Sponges + eCG**
  - ספוגיות + eCG (PMSG)
- **Prostaglandins**
  - פרוסטגלנדין
- **Melatonin**
  - מלטונין
- **Melatonin + Sponges + eCG**
  - מלטונין + eCG + ספוגיות
- **Sponges 6-7 days+ eCG + prostaglandins**
  - ספוגיות 6-7 ימים + eCG + פרוסטגלנדין
- **Melatonin + Prostaglandins**
  - מלטונין + פרוסטגלנדין

## Sponges + eCG

## ספוגיות + eCG (PMSG)



# Prostaglandins

# פרוסטגלנדינים

## סכרון בעזרת פרוסטגלנדינים SINCRONIZACIÓN CON PROSTAGLANDINAS<sup>3</sup>

1.<sup>a</sup> Inyección  
prostaglandina

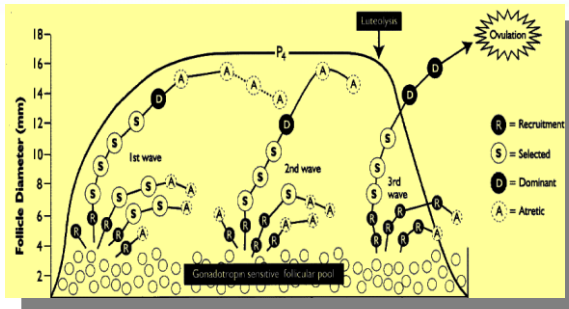
9-11 días

2.<sup>a</sup> Inyección  
prostaglandina

24 h

הרבעה

MONTA DIRIGIDA  
CUBRICIÓN



# Melatonin

# מלטונין

החדרת שתלים  
הוצאת איילים

הרבעה

IMPLANTS  
RAMS OUT

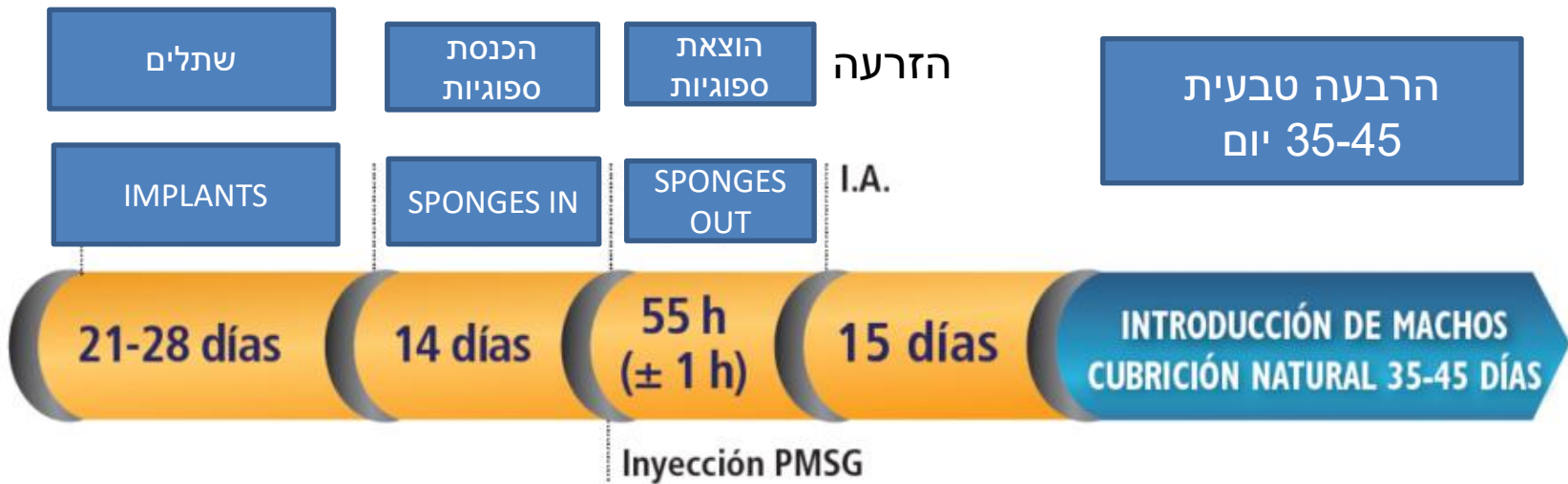
Introducción de machos  
Inicio de cubrición

35-42 días

RAMS IN



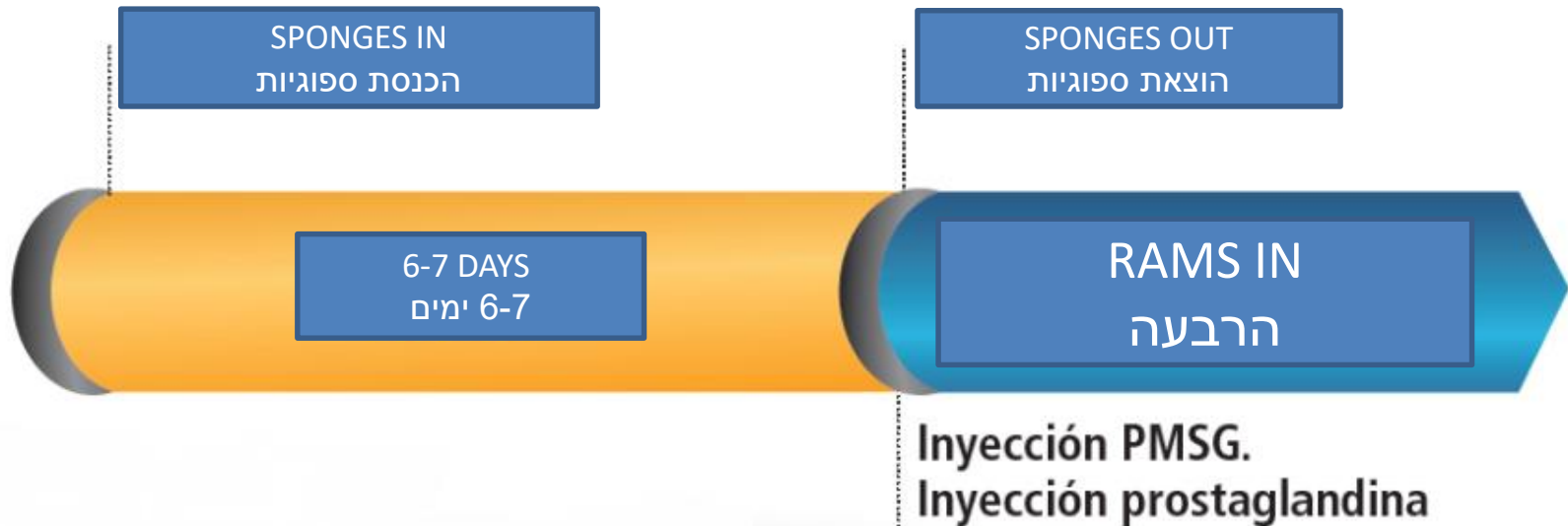
# Melatonin + Sponges + eCG מלטונין + eCG + ספוגיות





Sponges 6-7 days+ eCG + prostaglandins  
ספוגיות 6-7 ימים + eCG + פרוסטגלנדין

**PROTOCOLO CORTO PROGESTÁGENOS + PROSTAGLANDINAS<sup>2</sup>**



# Melatonin + Prostaglandins

## מלטונין + פרוסטגלנדינים

