A., Bassilly, S., and Darby, W.J., Am. J. clin. Nutr. *20* (1967) 422.
9 Magee, A.C., and Graiger, F.P., Nutr. Rep. Int. *29* (1979) 771.
10 Cossack, Z.T., Prasad, A.S., and Konuich, D., Nutr. Rep. Int. *26* (1982) 841.
11 Hackley, B.M., Smith, J.C., and Halsted, J.A., Clin. Chem. *14* (1968) 1.
12 Furlanetto, R.W., Underwood, L.E., Van Wyke, J.J., and D'Ercole, A.J., J. clin. Invest. *60* (1977) 684.
13 Snedecore, G.W., and Cochran, W.G., Statist. Meth. (1968) 59.
14 Momcilovic, B., Belonje, B., and Shah, B.G., Nutr. Rep. Int. *11* (1975) 445.
15 van den Brande, J.L., van Buul, S., Heinrich, V., van Reon, F., Zucher, T., and van Steirtegem, M., Adv. Metab. Disord. *8* (1975) 171.
16 Öner, G., and Bor, N.M., Nutr. Rep. Int. *18* (1978) 749.
17 Cheruvanski, T., Castro-Magana, M., Yao Chen. S., Collipp. P.J., and Maibodi, Z.G., Am. J. clin. Nutr. *35* (1982) 668.
18 Root, A.W., Duckett, G., Sweetland, M., and Reiter, E.O., J. Nutr. *109* (1979) 958.
19 Prasad, A.S., Oberlease, D., Wolf, P., and Horwitz, T., J. Lab. clin. Med. *73* (1969) 486.
20 Daughaday, W.H., Herington, A.C., and Phillips, L.S., A. Rev. Physiol. *37* (1975) 211.
21 Prasad, A.S., and Oberleas, D., J. Lab. clin. Med. *83* (1974) 83.

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## Do luteinized unruptured follicles secrete progesterone in mature female rats?[1]

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*Summary.* Unruptured luteinized follicles were shown to secrete less progesterone than did postovulatory corpora lutea in cyclic female rats.

It is now well established that the antiinflammatory agent indomethacin can inhibit ovulation by interfering with the synthesis of prostaglandins in preovulatory follicles[3-6], thus causing the formation of luteinized unruptured follicles (LUF) in hCG-treated mature rabbits[7] and in prepubertal gilts injected with PMSG and hCG[8]. Moreover, Ainsworth et al.[8] reported that LUF, formed in the gilt at the expense of preovulatory ovarian follicles, which had failed to rupture under indomethacin treatment, could secrete physiological amounts of progesterone. Previous work in our laboratory which showed that LH was capable, when injected on the day of dioestrus 2, of advancing ovulation by 24 h in 4-day cyclic female rats[9-11], encouraged us to determine whether indomethacin could prevent ovulation from occurring in cyclic female rats early exposed to the ovulatory action of LH. Pilot experiments justified this hypothesis indicating that combined LH and indomethacin treatment resulted in the formation of LUF. Experiments whose results are reported here were then carried out to study whether LUF displayed luteal activity in the rat.

*Material and methods.* 3-4-month-old virgin female rats bred in our colony (strain WI) were used. The animals were housed in a light- (lights on 23.00-13.00 h) and temperature- (22-24 °C) controlled room and given free access to food pellets and water. Following transfer from natural lighting conditions, the females were allowed a month to adapt to their new environment. Only those which had experienced 2 or 3 4-day cycles prior to experiments were used. Cycles consisted of dioestrus 1 and 2, prooestrus and oestrus.

The females were allocated to 2 groups. The first one served for the evaluation of the function of corpora lutea induced by LH on dioestrus 2 at 10.30 h. This corresponded to the clock-time (16.00-17.00 h) under natural lighting at which LH was either injected on dioestrus 2 in our preceding experiments or spontaneously released on proestrus in the WI strain of rats bred in our colony[12-14]. All the females were given a dose of 7.1 µg/100 g b.wt s.c. of LH M4 (LH M4 = 2.25 X NIH-LH-S3). Some of them received 5 mg/100 g b.wt i.v. of indomethacin by 6 h 30 min after LH administration. The 2nd group consisted of uninjected females which were used for the study of corpora lutea spontaneously developed during 4-day cycles. In all cases the females were killed by decapitation at 11.00 h, either on the expected day of estrus, in the LH-treated females or, on dioestrus 1, that is at the time of the highest activity of the corpora lutea[15], in the females running natural cycles. The ovaries were removed for histological examination. Postovulatory corpora lutea (POCL) and LUF were counted in each female. An ovulation coefficient (OC) was computed by dividing the number of POCL by the whole number of POCL and LUF. The mean number of POCL and LUF and a mean OC was calculated in each experimental procedure. Blood was collected for the determination of progesterone using a previously described RIA[16]. The data were analyzed by one-way analysis of variance and Scheffe's test. Progesterone values were studied following logarithmic transformation.

*Results and discussion.* As shown in the table the total number of corpora lutea comprising of both POCL and LUF did not differ in the rats with a natural cycle and in the LH-treated females ($F^2_{31} = 0.46$; NS). The number of LUF per animal appeared to be greater in LH + indomethacin-treated females than in those receiving LH only ($p < 0.05$). This was confirmed by comparing OC in both groups of animals: OC value was significantly lower in the former than in the latter ($p < 0.05$). Regarding the corpus luteum's function by 48 h after either LH release in natural cyclers or LH injection in treated animals we observed that blood progesterone concentration differed in the 3 groups of animals ($F^2_{31} = 17.78$; $p < 0.001$), with the highest value in those with a natural cycle and the lowest value in LH + indomethacin-treated animals as compared to LH-treated females ($p < 0.05$).

These data first confirm previous findings[17,18], showing that the pool of follicles available for ovulation on the day of prooestrus is present in the ovary as soon as the morning of dioestrus 2. It is probably the reason why the number of follicles sensitive to LH treatment on dioestrus 2 appeared to be statistically comparable to that of follicles able to ovulate under physiological circumstances. The main point which emerges from our observations concerns the ability of indomethacin to prevent ovarian follicles from rupturing on early exposure to the ovulatory action of LH, thus causing the formation of LUF. This is in agreement with the above mentioned observations in the rabbit[7] and in the gilt[8]. However LUF were shown to secrete less progesterone in the rat than did LH-induced and spontaneously formed POCL, by contrast with the gilt[8] in which LUF developed normal progesterone secretory activity. Indeed we chose to study LUF's function at a stage expected to correspond with the time of the highest activity of POCL in animals with a natural cycle. New experiments are then needed to establish the pattern of the functions of LUF at various times after their formation.

Luteal activity of LH-induced[a] postovulatory corpora lutea or luteinized unruptured follicles (LUF) and of spontaneous[b] postovulatory corpora lutea (POCL) in 4-day cyclic female rats

| Groups | | Number of animals | Total number of POCL and LUF | Number of POCL | Number of LUF | Ovulation coefficient | Blood progesterone concentration ng/ml |
|---|---|---|---|---|---|---|---|
| LH treatment on dioestrus 2[c] | Without indomethacin | 11 | 11.9 ± 0.9 | 9.0 ± 0.9* | 2.9 ± 0.6* | 0.77 ± 0.04* | 16.2 ± 1.5* |
| | With indomethacin[d] | 11 | 12.6 ± 0.8 | 3.5 ± 0.5** | 9.2 ± 0.9** | 0.28 ± 0.04** | 11.6 ± 2.2** |
| Natural cycle | | 12 | 12.9 ± 0.5 | 12.7 ± 0.5*** | 0.3 ± 0.1*** | 0.98 ± 0.01*** | 24.3 ± 1.2*** |

Values are the mean ± SE. [a] Sacrifice on the expected day of oestrus. [b] Sacrifice on the day of dioestrus 1. [c] 7.1 µg/100 g b.wt s.c. LH-M4 in equivalent of NIH-LH-S3 (LH-M4 = 2.25 X NIH-LH-S3) was injected at 10.30 h of an artificial light cycle with lights on 23.00–13.00 h. [d] 5 mg/100 g b.wt i.v. were given by 6 h 30 min after LH. ** $p < 0.05$ vs * and ***; *** $p < 0.05$ vs *.


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3 Le Maire, W.J., and Marsh, J.M., J. Reprod. Fert., suppl. *22* (1975) 53.
4 Espey, L.L., Biol. Reprod. *22* (1980) 73.
5 Espey, L.L., Biol. Reprod. *24*, suppl. 1 (1981) 46 A.
6 Espey, L.L., Coons, P.C., Marsh, J.M., and Lemaire, W.J., Endocrinology *108* (1981) 1040.
7 Bowring, N., Earthy, M., and Mangan, F.R., J. Endocr. *64* (1975) 11 P.
8 Ainsworth, L., Tsang, B.K., Downey, B.R., Baker, R.D., Marcus, G.J., and Armstrong, D.T., Biol. Reprod. *21* (1979) 401.
9 Aron, C., Anat. Anz., suppl. *109* (1960–61) 307.
10 Aron, C., and Asch, L., Archs Anat. microsc. Morph. exp. *49* (1960) 177.
11 Chateau, D., C.r. Acad. Sci. Paris *269* (1969) 788.
12 Hassani, M., Plas-Roser, S., Roos, J., and Aron, C., Acta endocr. *89* (1978) 551.
13 Geiger, J.M., Plas-Roser, S., and Aron, C., J. interdisc. Cycle Res. *12* (1981) 109.
14 Chateau, D., Plas-Roser, S., and Aron, C., Endokrinologie *77* (1981) 257.
15 Boehm, N., Plas-Roser, S., and Aron, C., Experientia *38* (1982) 1253.
16 Boehm, N., Plas-Roser, S., and Aron, C., J. Steroid Biochem. *16* (1982) 339.
17 Buffler, G., and Roser, S., Acta endocr. *75* (1974) 569.
18 Hirshfield, A., and Midgley, A.R., Biol. Reprod. *19* (1978) 597.


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## Season-dependent effects of melatonin on testes and fur color in mountain hares (*Lepus timidus* L.)[1]

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*Summary.* Melatonin was administered in 3 seasons to adult male mountain hares (*Lepus timidus* L.) kept in long or short photoperiods. Melatonin and short photoperiods induced testis regression and fur whitening in summer and autumn but not in winter. Both treatments combined seemed to provoke an advanced onset of the refractory period.

A large number of studies, mainly on rodents, have shown that the pineal hormone melatonin plays an important role in the regulation of annual rhythms that are controlled by the photoperiod, such as reproductive activity and change of fur color[3–5]. Findings in golden and djungarian hamsters indicated that the effect of administered melatonin depends on the photoperiodic conditions and on the phase of the annual cycle of the animals at the beginning of the experiment[6,7]. In order to test whether reactions to melatonin treatment similar to those observed in rodents could also be seen in lagomorphs, we carried out experiments with adult male mountain hares. This species shows marked seasonal rhythms in gonadal activity and fur color (brown in summer, white in winter) that are controlled by the photoperiod[8] and therefore possibly by melatonin released from the pineal gland.

*Materials and methods.* All hares, descendants of wild animals captured in Scandinavia, were purchased from the breeder Urogallo, Asiago, Italy (45.7° N, 1000 m). They had been kept outdoors for at least 1 year before the beginning of the experiments. During the experiments they were individually housed in special cages (for details see Spagnesi[9]). They were fed a standard diet (C2 EX, Palatamangimi, Bologna, Italy) and water ad libitum. The temperature was kept at 20 ± 4 °C; the humidity was about 70%. The only light was provided by fluorescent bulbs; the light intensity in the cages varied between 100 and 400 lx. Three experiments were carried out during different seasons; the first used animals in summer condition with large testes and brown fur, the second used animals in autumn condition with partly regressed testes and partial molt to white winter fur, the third was carried out with hares in complete winter condition with fully regressed testes and white fur. On July 6th, October 12th and January 8th the hares received either empty or melatonin-filled silastic tubes (i.d. 1.47 mm, 30 cm/animal, daily release about 150 µg/animal). The s.c. implantations were performed with the animals under anesthesia (Ketalar 50 mg/ml, Parke Davis). From the day of implantation half of each treatment group was kept in long (16 h light/day) while the remainder were kept in short (8 h light/day) photoperiods for 7 weeks. Changes in testis size were estimated once a week by palpation. Fur color was estimated from the regrowing fur on a plucked area on the back of the hare. At the end of each experiment the animals were killed and the testes removed, weighed and prepared for histological examination. As the number of animals per group was very limited (2–4)