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Perspectives and Editorials: Letters to the Editor

Artificial Vagina for Rabbits

To the Editor:

We read with interest ''Development of an Inexpensive Artificial Vagina for Semen Collection From Rabbits'' by Naughton et al (*J Androl.* 2003;24:712–715) and consider the underlying premise flawed. The abstract incorrectly states that ''no easily reproducible or inexpensive device for semen collection (from rabbits) has been reported.''

Prior to the mid 1960s, a ''Walton-type'' artificial vagina (AV) was used to collect rabbit semen. Amann and Lambiase (1967) and others provided a citation path leading to Macirone and Walton (1938). Each user had his or her own homemade version of this AV. For many years, IMV Technologies (www.imv-technologies.com) has sold a Walton-type AV; currently it costs $42.

The rabbit AV illustrated with instructions for fabrication by Bredderman et al (1964) rapidly gained wide acceptance because of convenient usage, minimal sperm loss, and low cost. It remains in use. A technician could fabricate a Bredderman-style AV from materials costing <$3.00 (including latex liner) plus $2.50 or $9.00 for a cutoff, 15-mL graduated centrifuge tube (polycarbonate or glass). This AV is easily washed and sanitized, so cost per ejaculate is very low.

Consistent quantitative recovery of ejaculated sperm is essential for most studies, yet Naughton et al did not estimate sperm loss in their AV. They implied that sperm loss in the collection condom might be substantial. Sperm loss within a Walton-type AV averaged 10% of the total number ejaculated (Amann and Lambiase, 1967), and loss averaged <4% within a ≤6-cm long Bredderman-style AV (Bredderman et al, 1964).

Considering semen collection, we never sedated a ''teaser doe''; <1 in 10 are/become obstinate and should not be used. A selection of female and male teasers is essential. The teaser should be placed into the male's cage rather than vice versa. Sexual preparation (ie, 3 false mounts; Macmillan and Hafs, 1967) is essential to maximize ''harvest'' of available sperm. False mounts are accomplished by ''brushing'' the male off the teaser several times before allowing ejaculation into the AV. Lack of sexual preparation likely contributed to low values for total sperm per ejaculate noted by Naughton et al. In our experiences (hundreds of males), urination concomitant with ejaculation is rare and usually results from too warm an AV. Even with proper temperature (43°C to 49°C), an occasional male urinates frequently; if feasible, he might be excluded from a study. Proper evaluation of a male requires multiple collections every 2 or 3 days, using false mounts, over a period of weeks or months (Amann and Lambiase, 1967; Foote et al, 1986).

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References

Amann RP, Lambiase JT Jr. The male rabbit. I. Changes in semen characteristics and sperm output between puberty and one year of age. *J Reprod Fertil.* 1967;14:329–332.

Bredderman PJ, Foote RH, Yassen AM. An improved artificial vagina for collecting rabbit semen. *J Reprod Fertil.* 1964;7:401–403.

Foote RH, Schermerhorn EC, Simkin ME. Measurement of semen quality, fertility, and reproductive hormones to access dibromochloropropane (DBCP) effects in live rabbits. *Fundam Appl Toxicol.* 1986;6: 628–637.

Macirone C, Walton A. Fecundity of male rabbits as determined by ''dummy matings.'' *J Agric Sci.* 1938;28:122–134.

Macmillan KL, Hafs HD. Semen output of rabbits ejaculated after varying sexual preparation. *Proc Soc Exp Biol Med.* 1967;125:1278–1281.

Response to Artificial Vagina for Rabbits

To the Editor:

We are grateful for the interests and comments of Drs Amann and Foote regarding our recent report of an artificial vagina assembled from inexpensive products purchased at a local Home Depot for the purposes of semen collection from rabbits. Andrologic studies using semen analyses from rabbits as a study endpoint have been reported for decades. However, most of these reports do not provide enough detail to reproduce the ''artificial vagina'' so widely quoted as a method of semen collection in rabbits. This report is simply a step-by-step description of how we overcame our obstacle of not being able to obtain

a commercial device to aid us in rabbit semen collection as an endpoint for a separate study. Following publication of this report, we have obtained several requests for article reprints by investigators using semen collection from rabbits as an endpoint. These requests are testimony to our frustrating experience in attempting to acquire even existing commercially available devices or to find a step-by-step description to reproduce a device from previous reports. Our goal in publishing this report is to provide an option for investigators who are involved in andrologic rabbit studies to reproducibly ''home'' construct an artificial vagina from easily obtainable products at low cost.

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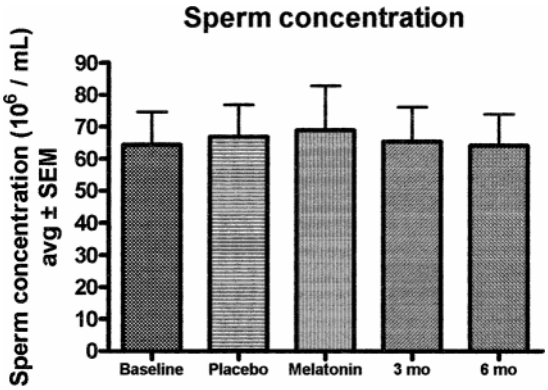
Melatonin Administration Alters Semen Quality in Normal Men

To the Editor:

The publication by Luboshitzky et al (2002) concerning the effects of melatonin on human sperm quality deserves strong critique for a number of reasons. The authors performed a double-blind crossover study during which healthy volunteers were given either melatonin (3 mg) or a placebo for 3 months each, while between the phases, a washout phase of 2 weeks was included. At the beginning and end of the 2 phases, a total of 11 parameters (sperm and endocrine) were measured and, again, 3 and 6 months after the end of the study. According to the results (!!), volunteers were divided into 2 groups, responders (n = 2) and nonresponders (n = 6). The criterion was that both sperm concentration and sperm motility ''dropped during the melatonin treatment period.'' Both of these men belonged to the group to which melatonin was given in the second treatment period. The title and the conclusions of this paper are simply not justified by the data for the following reasons:



Sperm concentration of all volunteers (n = 8) at the given condition. All differences are not significant (analysis of variance, $P = .998$). Original data were from figure 1A of the original publication.

1) It is certainly not correct to group the data according to the results. This procedure, when applied to whatever data set, will produce ''significant'' differences where no such differences exist. Moreover, in accordance with the authors' criterion, volunteers 2 and 8 would also be ''responders'' (however, in the opposite direction) because their sperm concentrations and sperm motility values were increased at the end of the melatonin treatment period. When looking at the mean values for sperm concentration, as extracted from the authors' drawings, no trend at all is seen (Figure). Thus, the original interpretation of the results is heavily skewed.
2) The 2 ''responders'' had the lowest baseline sperm concentrations of all volunteers, close to the lower limit as defined by the World Health Organization. Because of the high variability, most clinical trials involving sperm parameters as an endpoint have at least 2 baseline time points to exclude false-normal volunteers. This argument is underlined by the sperm concentrations observed in volunteer 3. Here, sperm concentrations under placebo treatment dropped and already are very close to 20 Mio/mL.
3) There is no information during which months the study was performed. It is known that sperm parameters vary significantly with the season (eg, Chen et al, 2003).
4) The serum E_2 values for the 2 responders were in the range of or even under the detection limit of the assay (44 pmol/L). Thus, these values as well as the derived T/E_2 ratio must be interpreted with caution.
5) It is well known that exogenous melatonin influences the diurnal rhythms of endogenous melatonin and of the whole circadian system (Lewy et al, 1998). Consequently, if hormones are analyzed that are known to be secreted in a diurnal fashion (Juneja et al, 1991), just 1 time point is insufficient for a reliable analysis.
6) In many animal species, melatonin is known to act through specific receptors on the hypothalamic, pituitary, and testicular levels. Despite the intense research, so far, melatonin receptors involved in human reproduction processes have not been identified.

In summary, I see no evidence whatsoever for the conclusion that melatonin impairs sperm parameters in healthy men. It rather appears that an expected result influenced the way the data were handled.

Sincerely,
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References

Chen Z, Toth T, Godfrey-Bailey L, Mercedat N, Schiff I, Hauser R. Seasonal variation and age-related changes in human semen parameters. *J Androl.* 2003;24:226–231.

Juneja HS, Karanth S, Dutt A, Parte P, Meherjee P. Diurnal variations and temporal coupling of bioactive and immunoactive luteinizing hormone, prolactin, testosterone and 17-beta-estradiol in adult men. *Horm Res.* 1991;35:89–94.

Lewy AJ, Bauer VK, Ahmed S, Thomas KH, Cutler NL, Singer CM, Moffit MT, Sack RL. The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. *Chronobiol Int.* 1998;15:71–83.

Luboshitzky R, Shen-Orr Z, Nave R, Lavi S, Lavie P. Melatonin administration alters semen quality in healthy men. *J Androl.* 2002;23:572–578.

Response: Melatonin Administration Alters Semen Quality in Normal Men

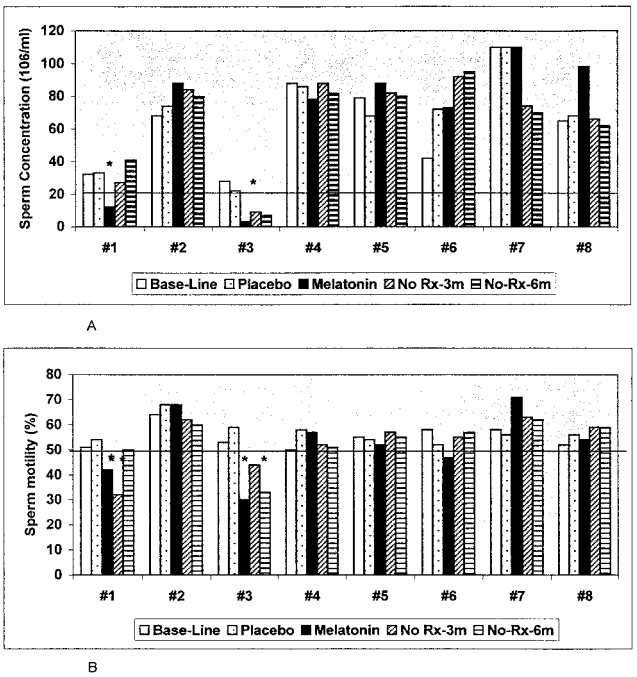
To the Editor:

We appreciate the comments of Lerchl (2004) regarding the effect of exogenous melatonin on sperm quality in normal men (Luboshitzky et al, 2002). In this study, we examined the possible effect of melatonin on semen concentration, motility, and morphology in 8 healthy young men. Since we did not study fertility in the general sense, we performed one semen analysis at baseline. We fully agree that 2 semen samples are indicated for the initial evaluation of fertility. In our study, we defined a subject as a responder if his sperm concentration and motility dropped during the melatonin treatment period. The individual results of semen analysis were given in our study in Figure 1. In 2 men, we found decreases in sperm concentration and motility that were below the normal range (WHO, 1993). Since the sequence of medications in these subjects was a placebo followed by melatonin, we concluded that the decrease in semen quality was associated with melatonin administration. We also observed an increase in sperm concentration in 3 subjects during melatonin administration. These counts were within the reference range and were not associated with similar changes in sperm motility. We attributed these changes to the well-known variations between samples that exist in the same individual (WHO, 1993). The data presented by Lerchl in the figure describe the average (±SEM) values for sperm concentration for all 8 volunteers examined. It is obvious from our study that, as a group, no trend is seen during melatonin treatment.

We also determined fasting serum gonadotropins and testosterone and estradiol levels. Although testosterone is secreted in a diurnal fashion (Luboshitzky et al, 2003), a single time point in the morning is sufficient for the assessment of the pituitary-gonadal axis function in men if hormone levels are within the reference range. A recent study has demonstrated that sperm parameters vary with season and advanced age (Chen et al, 2003). These results do not contradict our findings, as we conducted our study between October and May, in a different time zone and in young men.

We fully agree with the comment that exogenous melatonin influences the diurnal rhythm of the endogenous hormone. In fact, when suitably timed, melatonin administration appears to be beneficial in alleviating symptoms of circadian-based sleep disorders, shift work, jet lag, and delayed sleep phase syndrome as well as a sleep-promoting agent in elderly insomniacs (Zhdanova and Wurtman, 1997; Sack et al, 2000).

Taken together, the data suggest that exogenous melatonin alters semen quality in some men. Melatonin action at the hypothalamic-pituitary level is less likely in view of unaltered serum gonadotropin levels in our study. A direct inhibitory effect of melatonin on testicular and epididymal



Sperm concentration **(A)** and motility **(B)** in 8 healthy men during 6 months of melatonin-placebo administration. The solid line represents the lower normal limit suggested by World Health Organization guidelines (1993). The sequence of medications was a placebo followed by melatonin for subjects 1–4 and melatonin followed by a placebo for subjects 5–8. All 8 subjects had a 2-week washout period after the first 3-month treatment period. Statistically different values are indicated with an asterisk.

aromatase resulting in an altered androgen/estrogen milieu and, consequently, decreased sperm concentration and motility is a more plausible possibility. This is supported by previous observations that low sperm production is associated with low seminal plasma aromatase activity and higher melatonin levels (Yie et al, 1991). Also, in frogs, melatonin has a direct inhibitory effect on the basal- and estradiol-stimulated mitotic activity of primary spermatogonia in the testis (d'Istria et al, 2003).

In summary, the results of our preliminary study suggest that melatonin decreases sperm counts and motility to subnormal levels in some healthy young men. When considering the long-term use of melatonin, extra precautions should be taken, especially in men with low normal sperm counts.

Sincerely,
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References

Chen Z, Toth T, Godfrey-Bailey L, Mercedat N, Schiff I, Hauser R. Seasonal variation and age-related changes in human semen. *J Androl.* 2003;24:226–231.

d'Istria M, Palmiero C, Seino I, Izzo G, Minucci S. Inhibition of the basal and oestradiol-stimulated mitotic activity of primary spermatogonia by melatonin in the testis of the frog, Rana esculenta, in vivo and in vitro. *Reproduction.* 2003;126:83–90.

Lerchl A. Letter to the editor. *J Androl.* 2004;25:185.

Luboshitzky R, Shen-Orr Z, Herer P. Middle-aged men secrete less testosterone at night than young healthy men. *J Clin Endocrinol Metab.* 2003;88:3160–3166.

Luboshitzky R, Shen-Orr Z, Nave R, Lavi S, Lavie P. Melatonin administration alters semen quality in healthy men. *J Androl.* 2002;23:572–578.

Sack RL, Brandes RW, Kendall AR, Lewy AJ. Entrainment by free-running circadian rhythms by melatonin in blind people. *N Engl J Med.* 2000;343:1070–1077.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Semen–Cervical Mucus Interactions.* New York, NY: Cambridge University Press; 1993.

Yie SM, Daya S, Brown GM, Deys L, Younglai EV. Melatonin and aromatase stimulating activity of human seminal plasma. *Andrologia.* 1991;23:227–231.

Zhdanova IV, Wurtman RJ. Efficacy of melatonin as a sleep-promoting agent. *J Biol Rhythms.* 1997;12:644–650.