Induction of Precocious Maturation of Spermatogenesis in Infant Rats by Human Menopausal Gonadotropin and Inhibition by Simultaneous Administration of Gonadotropins and Testosterone*

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ABSTRACT. This study was undertaken to determine if the initiation of spermatogenesis could be modified by the administration of gonadotropins and sex hormones in infant rats. Five-day-old rats were injected daily between the 5th and 11th days of life with test substances and killed on day 15. Administration of testosterone propionate (TP; 2.5 mg daily), human menopausal gonadotropin (hMG; 7.5 IU daily), or coadministration of both of these substances (TP + hMG) or administration of estradiol benzoate (15 μg daily) caused quantitative changes in premeiotic spermatogenesis, as measured by the mean cell counts per tubule cross-section.

hMG caused an increased yield of type A, spermatogonia (SgA) from undifferentiated type A spermatogonia (UnA) and increased the yield of type B spermatogonia from SgA. TP was not effective in stimulating first premeiotic spermatogenesis, and in contrast to hMG, it had a negative influence on the numbers of UnA and SgA, and on the volume of Sertoli cell nucleus. Administration of TP + hMG or estradiol benzoate resulted in a significant increase in the numbers of UnA and SgA, but inhibited cell differentiation. TP + hMG significantly reduced the rate of premeiotic spermatogenesis.

The results demonstrate that precocious numerical stabilization of premeiotic spermatogenesis can be achieved by the application of hMG. TP applied alone was able to induce peripheral androgenic effects (seminal vesicle weight) 100% greater than those produced by administration of hMG, but was not able to stimulate seminal tubule function. TP applied together with hMG produced inhibition of spermatogenesis. This effect might be due to the inhibition of Sertoli cell function by the direct influence of testosterone. In contrast to testosterone, estradiol may play a stimulatory role in the multiplication of the reserve stem cells of the first spermatogenesis of the rat. (Endocrinology 122: 34–39, 1988)

QUALITATIVE examination of the first spermatogenesis in the rat revealed that the first meiotic event proceeds together with increased biosynthesis of androgens by testicular tissue in vitro (1). The yield of premeiotic steps, i.e., formation of preleptotene spermatocytes, was quantitatively impaired after selective withdrawal of FSH (2). It has been suggested, however, that the earliest steps initiating the spermatogenic process [i.e., the transformation of undifferentiated spermatogonia into type A, spermatogonia (SgA)], could not be influenced by androgens (3) or FSH (2).

In the rat, very early postnatal development is associated with relatively high serum concentrations of both FSH and LH. In further development, between about 20th and 60th days after birth, a marked rise of serum FSH is noted (4). FSH alone, however, does not seem to be responsible for the initiation of seminal tubule function. Moger (5) has demonstrated that continuously high FSH levels induced by hemichidectomy did not result in advance of puberty onset. The aim of the present study was to investigate the possibility of inducing precocious initiation of spermatogenesis with human menopausal gonadotropin (hMG), testosterone propionate (TP), and both of these agents (TP + hMG).

Materials and Methods

Animals

Five-day-old male Wistar rats, born on the same day, were divided into five groups of five to eight animals. Each group was kept in a separate cage together with an adult female; animals were fed throughout the experiment.

Adult females were fed with regular pellets, carrots, milk, and water ad libitum. The animal room provided stable diurnal light-dark cycles (light phase, 0800–2000 h) and stable temperature.

Infant rats were injected daily, beginning on the 5th day after birth until the 11th day, with 2.5 mg TP-Polfa (TP group), with 7.5 IU Humegen-Orgonon (hMG group), both TP and
hMG (TP + hMG group), 15 μg estradiol benzoate-Polfa (E₂B group), or olive oil (control group). On the 15th day of life the rats were killed with an overdose of chloroform inhalation. The weights of the body, paired testes, and paired seminal vesicles were measured at autopsy. One testis of each rat was fixed in Bouin fixative and processed for regular histology. Eight-micron thick sections from equatorially transected organs were stained with periodic acid-Schiff reagent and counterstained with hematoxylin.

Quantitation of testis function

Clermont and Perey (6) demonstrated that in rats, the seminiferous epithelium cycle is already established beginning from the 15th day of age. Huckins (7) showed that A spermatogonia (SgA), which can be identified among B spermatogonia (SgB; stages V-VII), are undifferentiated type A spermatogonia (UnA). UnA metabolise, without cell divisions into SgA. In the subsequent stages (VII-VIII) 80-90% of SgA are actually of SgA (7, 8).

In the present study spermatogenesis was assessed by determining the number of UnA, SgA, SgB, and preleptotene spermatocytes. Identification of UnA was based on their location among SgB and on the morphology of their nucleus. This nucleus is less basophilic than that in the differentiated spermatogonia and contains few or no heterochromatic clumps or granules and small nucleolus. SgA were identified as the differentiated spermatogonia located among preleptotene spermatocytes in the tubules containing the association of cells corresponding to stages VII-VIII of the seminiferous epithelium cycle (7-9).

A projection microscope was used. Each germinal cell type and Sertoli cells were separately marked on a glass Fresnell-frosted screen (Carl Zeiss, Jena, DRG) and recorded. Each rat was represented by means of cell counts from 20 circular cross-sections of tubules containing SgB and 20 tubules containing preleptotene spermatocytes. All cell types were expressed per 1 tubular cross-section.

The counts were corrected for differences in nuclear diameter (10) and adjusted for tubular shrinkage by using a Sertoli cell correction factor (11, 12). The number of Sertoli cells was corrected with respect to changes in tubular area and changes in the volume of the nucleus (12). The means of the relative number of each type of germinal cells were compared between groups using Student’s t test.

An advance of the progression of first spermatogenesis was determined semiquantitatively by measuring the frequency of occurrence of the tubules containing the most mature germinal cells. The mean number of tubules containing more than 3 pachytene/100 transsections examined represented each rat (13). The mean percentages calculated for the groups were compared using Student’s t test.

Results

Organ weight

The mean body weight was significantly higher than that in the control group after treatments with TP, hMG, and E₂B but not after treatment with TP + hMG.

The weight of seminal vesicles was significantly stimulated by the administration of hMG and was almost 2-fold higher than that of the control group. TP administration produced a 4-fold increase in the weight of the seminal vesicles, but combined treatment with TP and hMG resulted in partial elimination of this effect (Table 1).

The weight of the testes was increased more than 2-fold by the administration of hMG. The combined administration of TP and hMG resulted in partial elimination of this effect (Table 1).

Histology of the seminal tubules

Neither one of the treatments induced qualitative changes in the seminiferous epithelium, but single degenerative cells were found in the center of a few tubules of the testis in TP and E₂B groups. It was not possible to accurately identify these cells.

The mean diameter of the seminal tubule was significantly increased after TP or hMG treatment and significantly reduced after TP + hMG administration (Table 2).

Spermatogenesis did not pass through the pachytene stage of primary spermatocytes in all groups of rats. However, there were significant differences in the frequency of occurrence of the tubular cross-sections containing the most advanced cell types. The mean percentage of tubules with spermatogenesis advanced to pachytene spermatocytes was significantly higher than the control value in the animals treated with hMG alone and in animals treated with E₂B. Combined administration of TP with hMG resulted in a significant reduction of this parameter (Table 2).

Treatment with TP resulted in a significant decrease in the mean volume of the nuclei of the Sertoli cells. The combined treatment with TP and hMG partially reversed this effect (Fig. 1).

Table 1. Body weight, paired seminal vesicle weight, and weight of paired testis of immature rats treated with TP, hMG, TP + hMG, or E₂B.

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>Seminal vesicle wt (mg)</th>
<th>Wt of testes (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>15.52 ± 0.82</td>
<td>5.28 ± 1.50</td>
<td>42.57 ± 2.82</td>
</tr>
<tr>
<td>hMG (5)</td>
<td>20.61 ± 1.01*</td>
<td>20.83 ± 2.99*</td>
<td>99.66 ± 12.09</td>
</tr>
<tr>
<td>TP (6)</td>
<td>18.54 ± 1.27*</td>
<td>9.57 ± 1.62*</td>
<td>106.85 ± 10.88*</td>
</tr>
<tr>
<td>TP + hMG (5)</td>
<td>17.06 ± 2.83</td>
<td>11.66 ± 2.58*</td>
<td>74.33 ± 9.99*</td>
</tr>
<tr>
<td>E₂B (7)</td>
<td>19.44 ± 0.84*</td>
<td>9.75 ± 1.66*</td>
<td>45.50 ± 4.50</td>
</tr>
</tbody>
</table>

The number of animals is in parentheses; values are the mean ± SD. Significance was determined by Student’s t test.

* P < 0.001 vs. control group.
* P < 0.01 vs. control group.
* P < 0.05 vs. control group.
* P < 0.01, TP + hMG-treated vs. hMG-treated animals.
TABLE 2. Diameter of seminal tubules and percentage of tubules containing pachytene spermatocytes in immature rats treated with TP, hMG, TP + hMG, or E₂B

<table>
<thead>
<tr>
<th>Group</th>
<th>Seminal tubule diameter (μm)</th>
<th>% Tubules containing pachytene spermatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (8)</td>
<td>93.1 ± 3.72</td>
<td>19.5 ± 7.41</td>
</tr>
<tr>
<td>TP (5)</td>
<td>99.5 ± 1.13</td>
<td>20.1 ± 9.06</td>
</tr>
<tr>
<td>hMG (6)</td>
<td>100.7 ± 5.28</td>
<td>95.0 ± 5.28</td>
</tr>
<tr>
<td>TP + hMG (5)</td>
<td>85.6 ± 0.83</td>
<td>6.5 ± 2.98</td>
</tr>
<tr>
<td>E₂B (7)</td>
<td>95.4 ± 2.81</td>
<td>34.7 ± 5.04</td>
</tr>
</tbody>
</table>

The number of animals is in parentheses; values are the mean ± SD. Significance was determined by Student’s t test.

* P < 0.05 vs. control group.

# P < 0.01 vs. control group.

## P < 0.001 vs. control group.

### P < 0.001, TP + hMG-treated vs. TP-treated animals.

# Sertoli nucl. vol. (μm³)

![Image of Sertoli cell nuclei volume](image)

**Fig. 1.** Mean (±SD) volume of Sertoli cell nuclei in 15-day-old rats treated between the 5th and 11th days of life with TP, hMG, TP + hMG, or E₂B. Calculations were made using the formula 4/3 ab², where a is half the maximum nuclear length, and b is half the maximum nuclear width (12). In each rat 50 nuclei were measured. # P < 0.05, compared with control group (C); b, P < 0.05, TP + hMG compared with TP alone-treated animals (by Student’s t test).

**Function of the seminiferous epithelium**

Table 3 presents the mean relative germinal cell number under the influence of different treatments. Changes induced by TP were not significant with respect to the control group values, but a decrease in the mean number of UnA and SgA₁ was noticeable, and it occurred in this group only. hMG increased the number of SgB (# P < 0.001) and the number of preleptotene spermatocytes (# P < 0.001).

<table>
<thead>
<tr>
<th>Group</th>
<th>UnA</th>
<th>SgA₁</th>
<th>SgB</th>
<th>Preleptotene spermatocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.57 ± 0.51</td>
<td>2.25 ± 0.70</td>
<td>8.03 ± 1.87</td>
<td>15.72 ± 4.14</td>
</tr>
<tr>
<td>TP</td>
<td>1.03 ± 0.20</td>
<td>1.58 ± 0.20</td>
<td>8.43 ± 1.20</td>
<td>17.83 ± 2.20</td>
</tr>
<tr>
<td>hMG</td>
<td>1.54 ± 0.20</td>
<td>2.78 ± 0.38</td>
<td>18.77 ± 3.71</td>
<td>39.60 ± 5.88</td>
</tr>
<tr>
<td>TP + hMG</td>
<td>2.08 ± 0.94</td>
<td>3.00 ± 0.75</td>
<td>6.85 ± 1.50</td>
<td>11.67 ± 4.90</td>
</tr>
<tr>
<td>E₂B</td>
<td>2.68 ± 0.64</td>
<td>3.15 ± 0.71</td>
<td>9.40 ± 2.57</td>
<td>18.17 ± 5.23</td>
</tr>
</tbody>
</table>

All cell counts were corrected for differences in nuclear diameter by Abercrombie’s formula (10) and corrected for tubular shrinkage by a Sertoli cell correction factor (12). Values are the mean ± SD. Significance was determined by Student’s t test.

* P < 0.001 vs. control group.

# P < 0.01, TP + hMG-treated vs. TP-treated animals.

## P < 0.05 vs. control group.

### P < 0.05, TP + hMG-treated vs. TP-treated animals.

#### P < 0.001, TP + hMG-treated vs. hMG-treated animals.

### P < 0.01 vs. control group.

of UnA and SgA₁ was noticeable, and it occurred in this group only. hMG increased the number of SgB (# P < 0.001) and the number of preleptotene spermatocytes (# P < 0.001).

Treatment with TP + hMG resulted in an elevation in the number of SgA₁ compared with the value when TP was given alone (# P < 0.01). The lowest numbers of SgB and preleptotene spermatocytes were observed in the TP + hMG group. The number of SgB was significantly lower than that in controls (# P < 0.05) or rats treated with TP alone (# P < 0.05). The number of preleptotene spermatocytes was significantly lower in TP + hMG-rats than in rats treated with hMG alone (# P < 0.001).

A significant increase in UnA number with respect to the control value was noted in animals injected with E₂B (# P < 0.01). This was also found in the number of SgA₁ (# P < 0.05), but not in SgB or preleptotene spermatocytes (Table 3).

The ratios of germinal cells presented in Table 4 was derived from the mean values shown in Table 3. These ratios can be compared to those obtained by other investigators (6, 11, 14), because in all instances germinal cell numbers were corrected for differences in the diameter of the nucleus and in the thickness of the histological slide. A correction for shrinkage of the tubules was also made, as described by Clermont and Morgenstal (11) and modified by Lino (12). The later types of methodological differences might influence only the numbers of germinal cells per tubular cross-section, not the ratios between them.

Table 4 indicates that treatment with TP, which resulted in a relative lowering of the numbers of UnA and SgA₁ (Table 3), did not impair the efficiency of the differentiation of UnA into SgA₁. Similarly, hMG treatment, which did not significantly increase the number of
Table 4. Ratios of the mean relative numbers of the germinal cells in immature rats treated with TP, hMG, TP + hMG, or E\textsubscript{2}B

<table>
<thead>
<tr>
<th>Group</th>
<th>UnA/SGa\textsubscript{1}</th>
<th>SgA\textsubscript{2}/SGb</th>
<th>UnA/SGb</th>
<th>UnA/preleptotene\textsuperscript{a}</th>
<th>SgB/preleptotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.43</td>
<td>1.53</td>
<td>1.51</td>
<td>1.10</td>
<td>1.28</td>
</tr>
<tr>
<td>TP</td>
<td>1.13</td>
<td>1.55</td>
<td>1.82</td>
<td>1.17</td>
<td>1.22</td>
</tr>
<tr>
<td>hMG</td>
<td>1.33</td>
<td>1.67</td>
<td>1.20</td>
<td>1.25</td>
<td>1.21</td>
</tr>
<tr>
<td>TP + hMG</td>
<td>1.34</td>
<td>1.18</td>
<td>1.57</td>
<td>1.27</td>
<td>1.27</td>
</tr>
<tr>
<td>E\textsubscript{2}B</td>
<td>1.17</td>
<td>1.50</td>
<td>1.35</td>
<td>1.67</td>
<td>1.92</td>
</tr>
</tbody>
</table>

12-day-old intact rat\textsuperscript{b} 1:6.4 1:7.9 1:1.2
26-day-old intact rat\textsuperscript{b} 1:12.5 1:25.6 1:2.0
Adult intact rat\textsuperscript{c} 1:12.2 1:26.3 1:2.1
Adult hypophysectomized rat\textsuperscript{d} 1:5 1:26 1:2.0

\textsuperscript{a} Preleptotene spermatocytes.
\textsuperscript{b} From Clermont and Perey (6).
\textsuperscript{c} From Clermont (14).
\textsuperscript{d} From Clermont and Morgentaler (11).

SgA\textsubscript{2}, stimulated the differentiation of UnA into SgA\textsubscript{2}. Although E\textsubscript{2}B stimulated the numbers of UnA and SgA\textsubscript{2}, the yield of SgA\textsubscript{2} from UnA was reduced.

The step that most readily responded to the treatment employed was transition between SgA\textsubscript{2} and SgB. At this point both TP and hMG were effective stimulators, while TP + hMG caused inhibition. It must be emphasized that the passage between SgA\textsubscript{2} and SgB contains four intermediate steps of spermatogenesis, represented by type A spermatagonia differentiation (A\textsubscript{3}, A\textsubscript{4}, and A\textsubscript{5}) and by intermediate type spermatagonia (7, 8). Thus, one or more steps could have been influenced.

Neither of the treatments applied was capable of changing the ratio between SgB and preleptotene spermatocytes. It appears that, as in the controls, this ratio also changes with maturation, but becomes stable somewhere between the 12th and the 25th days of age. It can also be seen that hMG accelerated the numerical efficiency of premeiotic spermatogenesis to the values reported in 26-day-old immature rats, i.e., when meiotic division is already completed (6), and the values reported in adult rats. In contrast, both TP + hMG and E\textsubscript{2}B caused a reduction in the yields of cell differentiation comparable to those observed in hypophysectomized adult rats (Table 4).

Discussion

The results presented here indicate that premeiotic spermatogenesis in infant rats can be stimulated by hMG to precociously achieve a mature appearance. Equilibration of FSH-LH biological potencies in the hMG preparation was documented by the finding that both seminal vesicle weight and the weight of the testes were increased to the same extent (both doubled) in hMG-treated rats with respect to the control values. The first parameter represents androgen potency (15) and, indirectly, the bioactivity of LH, and the second one represents the activity of exogenous FSH (16).

Androgen effects achieved by the administration of TP alone were at least 2 times higher than those achieved by means of hMG administration, as measured by the mean weight of the seminal vesicles. Within the testes, TP alone led to an increase in the diameter of the seminiferous tubules and an insignificant stimulation of the number of preleptotene spermatocytes. The increase in the ratio between SgB and SgA\textsubscript{2} was relative, since TP inhibited the number of UnA and SgA\textsubscript{2}.

The administration of TP produced a significant fall in the mean volume of the Sertoli cell nucleus, which was also marked in animals treated with TP + hMG. This parameter may serve as an indicator of the inhibition of cell function (17).

Although TP given alone did not impair the initiation of spermatogenesis, it did so in animals treated simultaneously with TP + hMG. During the latter treatment, newborn rats were chronically exposed to an unequivocally stimulated, favoring androgen action. Unexpectedly, this treatment resulted in both the elimination of FSH-dependent stimulatory effects (testicular weight) and the reduction of the androgenic effects produced by TP given alone.

The first negative effect could be explained in part by the proposed inhibition of Sertoli cell function, but this explanation is not sufficient since TP alone did not impair spermatogenesis. It has to be stressed that a similar trend of changes in the yields of cell differentiation, but without effective inhibition of the rate of spermatogenesis, occurred after treatment with E\textsubscript{2}B. The coadministration of testosterone and gonadotropins produces increased conversion of exogenous testosterone into estrogen within the testis (18). Presumably, both the direct inhibitory effect of testosterone on Sertoli cell function and the increased metabolism of intratesticular
testosterone might account for the inhibition of first spermatogenesis in TP + hMG-treated rats. Kotite et al. (19) demonstrated that while testosterone or LH treatment enhanced the effect of FSH on androgen-binding protein accumulation in the immature rat testis, they completely blocked FSH effect on androgen-binding protein secretion.

The mechanism by which combined administration of hMG+TP decreased manifestation of the androgenic potency of exogenous testosterone is not easily explained. Increased aromatization or formation of other testosterone metabolites might result in a deficiency in testosterone availability. An increase in sex hormone-binding globulin and/or a decrease in androgen cytosol receptors can be equally considered.

Overall, our data suggest that factors that are generally considered as stimulatory may negatively influence seminiferous epithelium function, and that this influence can be direct in nature. The possibility of the direct negative regulation of Sertoli cell function by testosterone would be in contrast to the general view that testosterone negatively affects immature rat spermatogenesis only by means of the inhibition of gonadotropin secretion (20). Our results may confirm the early observations of Mancini (21) that testosterone given together with hMG to hypophysectomized men had an inhibitory effect on the response to hMG therapy.

In contrast, estradiol, a hormone that is generally considered to be inhibitory to testicular function may not have a direct inhibitory role in the progression of testicular development. It has to be taken into consideration, however, that exogenous estrogen may be less effective than estrogen produced endogenously. Exogenous estrogen is less efficient in the negative regulation of gonadotropin secretion in prepubertal rats (22) as well as in its negative influences within testes (23).

The data presented here provide some insight into the mechanism and hormonal control of the initiation of spermatogenesis. Stimulation by gonadotropins consisted of an increase of the yields of both SgA1 from UnA and SgB from SgA1. The transition between SgB and preleptotene spermatocytes was the step that could not be effectively stimulated or inhibited by the procedures used. It appears that the divisions of SgB are less dependent on hormones, since after hypophysectomy the ratio between SgB and preleptotene spermatocytes has an optimal theoretical value of 1:2 (11).

It can be assumed that the specific locus important for the final numerical acceleration and precocious matura tion of premeiotic spermatogenesis is the enhancement of differentiation of the earliest step examined, i.e. the metamorphosis of UnA into SgA1. The only locus where the stimulatory effect of testosterone could also be considered was the transition between SgA1 and SgB. The increased yield of SgB after treatment with TP can also be deduced from the results of Chemes et al. (20).

Differentiation of UnA into SgA1 was previously thought to be unaffected by the hormones (2, 3). The results presented here suggest that this step requires gonadotropin action. The statement of Chemes et al. (2) that FSH is required for one or more steps between SgA1 and preleptotene spermatocytes is supported by the findings mentioned above. Namely, it is unlikely that LH and androgens were responsible for the increased transition between UnA and SgA1, since TP given alone did not influence this step.

The data presented here showed that a short duration of testosterone administration to infant rats older than 4 days of age did not reduce testicular weight or inhibit early spermatogenesis. Steinberger and Duckett (24) demonstrated that testicular growth is significantly impaired when rats are treated with TP continuously, beginning from the day of birth; this could be noted in animals younger as well as older than 15 days of age. The discrepancy with our findings may arise from the fact that the treatments used here omitted the most critical period of testicular development in rats, namely the first day after birth. The administration of testosterone or estradiol on this day results in a permanent reduction of testis weight, which apparently is not mediated by the changes in gonadotropin secretion, but, rather, depends on its direct action on the testis (25).

Beside quantitative changes in premeiotic spermatogenesis, hMG induced an advance in its first progression as more tubules contained cells initiating meiosis (i.e. pachytene spermatocytes). This was not observed after TP administration, but unexpectedly was present after the application of E2.B. It seems that the enhanced spreading of the cells representing progression of the first meiosis event was a direct consequence of the increased pool of cells preceding pachytene formation.

An increase in the number of SgA1 caused by E2.B could be from a direct effect of estrogen on the testes. Firstly, this could not be produced by hMG alone, but was present after TP + hMG administration. Secondly, stimulation of UnA divisions by estrogen in adult mice was observed by De Rooij (26), who interpreted it as a compensatory mechanism in the face of inhibition of cell differentiation.

An increase in the number of SgA1 was observed after a decline in the intratesticular testosterone concentration (27). These researchers could not maintain a normal germinal cell number even with a 30-fold increase in serum testosterone produced by testosterone implants. Significant changes in the quality and quantity of spermatogenesis were observed by them in the presence of comparable serum FSH and testicular testosterone levels. They concluded that factors other than gonadotro-
pins and testosterone are involved in the regulation of spermatogenesis. Their study did not take into consideration the role of aromatization of exogenous androgen.

It is possible that estrogens may play some stimulatory role in the initiation of spermatogenesis by means of increasing the number of germinal cell precursors, including UnA. It has been shown that aromatase activity in newborn rats is localized exclusively within seminal tubules and undergoes sharp diazlocation into Leydig cells on the 15th day after birth (28). Maximal capacity of FSH for stimulating estrogen synthesis is also limited to the initial 12 days of life (29). Most recent results indicate that prenatal treatment with estrogen in the mouse accelerates prespermatogenesis. At the same time, the morphology of Leydig cells suggests an impaired biosynthesis of testosterone (30). Further studies are necessary to elucidate the role of estrogen in the development of the testis.

Acknowledgments

I wish to express my sincere thanks to Dr. H. Orłowska from Organon International BV (Oss, Holland; representation Warszawa, for the gift of hMG preparation (Humecon-Organon); to Dr. D. Pietrowski from the Institute of Occupational Medicine in Lodz for housing the animals; and to Prof. Dr. M. Pawlikowski from the Institute of Endocrinology in Lodz and Prof. Dr. F. Leidenberger from the Institute of Reproduction in Hamburg for their invaluable help.

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