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Morphometry and Ultrastructure of Stage IX-Specific Effects on Rat Sertoli and Spermatogenic Cells Immediately After 7-Day Testosterone Treatment in One Group and the Same Treatment in Another Group Followed by 7-Day Non-Treatment

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Abstract

Purpose: In our previous study, morphometric and ultrastructural analysis of stage-specific effects of Sertoli and spermatogenic cells were seen immediately after 7-day testosterone treatment (T group) in rat testes. The results strongly suggested that significant regulatory factors in spermatogenesis remain to be discovered. The present study was conducted to determine the morphometric and ultrastructural analysis of rat sertoli and spermatogenic cells in stage IX T group in one group and the same treatment in another group followed by 7-day non treatment (AT group), and also to assess whether or not the suggested unknown regulatory factors exist in the AT group.

Results: In the AT group, concentrations of testosterone decreased more prominently than in its counterpart in the T group. However, its concentration was lower than its counterpart in the control group of rats. Both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the AT group increased significantly more than their counterparts in the T group. Further they were closer to their counterparts in the control group than their counterparts in the T group.

The absolute volumes of seminiferous tubules in the AT group increased to levels closer to their counterparts in the control group than to their counterpart in the T group. In the cytoplasm of the Step 9 spermatid in the T group, disorganization of the microtubules in a manchette-like structure appeared. However, they assumed a normal manchette-like orientation in the AT group. The fine structures corresponding to the transverse section of tails in normal spermatozoa were detected in the adluminal region of the seminiferous epithelium in the AT group, and these findings suggest that normal spermatozoa were formed in the AT group.

Conclusion: The present study strongly suggests that unknown regulatory factors remain to be discovered.

Keywords: Morphometry; Ultrastructure; Sertoli cell; Spermatogenic cell; Testosterone; Stage IX; Rat

Introduction

In our earlier study [1], the T group in the Step 9 spermatid had disoriented microtubules within manchette-like structures. However, the relation and roles of both T and FSH with these abnormalities related to spermatogenesis could not be explained based on alterations in both T and FSH in peripheral blood. In Sertoli cells at stage IX, the androgen receptor is absent [2], and the mRNA of the FSH receptor in Sertoli cells is at a minimum in stage IX after a robust peak in both VII and VIII stages [3,4]. Even though LH remained lower in the T group than in the control group, testosterone was high enough to stimulate androgen receptors in the Sertoli cells. Furthermore, unexpected shifts in morphometry between the control group and T group in several parameters, as well as the appearance of disorganized manchette-like microtubules in the type 9 spermatids in the T group [1], could not be explained on the basis of alterations in T, LH, and FSH, since their respective receptors were absent in Sertoli cells in stage IX. Also, in the baso-lateral cytoplasm in Sertoli cells near the inter-Sertoli tight junction, mitochondria are closely opposed by the cisternae of RER at the outer membrane of the ultrastructure. This suggests that an active protein synthesis stimulated by certain factors takes place without the hormones mentioned above. These results suggest that still unknown regulatory factors are apparently involved in the developmental interactions between Sertoli and spermatogenic cells. The present study was conducted to determine the morphometry of rat Sertoli and spermatogenic cells in stage IX immediately after 7-day testosterone treatment in one group and the same treatment in another group followed by 7-day non treatment, and also to assess whether or not the suggested unknown regulatory factors [1] exist in the AT group.

Materials and Methods

Nine rats (Wistar strain) aged 12 weeks were separated into 3 groups; 3 rats for the control group, 3 rats for the T group, and the remaining 3 rats for the AT group. This third group was sampled after 7 days of no testosterone treatment. For testosterone treatment, rats

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were dorsally injected subcutaneously daily with 1 mg of testosterone propionate, as shown in the Figure 8.

The testosterone treatment was designed to suppress LH-dependent factors such as androgens from Leydig cells, and to cause a lack of stimulation of spermatogenic cells by Sertoli cells. Plasma concentrations of the hormones were measured from peripheral blood with the radioimmunoassay method [5] as in the former study [1].

Fixation of testes and tissue processing was already described [1,6,7]. Identification of the stages of the cycle was made on the basis of Hess [8]. The sampling and morphometric analyses were performed according to standard methods [1,6,9-11]. The numerical results were given a ± standard error of the mean (SEM), and the probability of the values was evaluated by Student's t-test.

Abbreviations for Morphometric Terms

Abbreviations were cited from references [10,11] as follows: SC (Sertoli cell); SN (Sertoli cell nucleus); VSC (μm^3), absolute volume of an average single Sertoli cell; VSN (μm^3), absolute volume of an average single Sertoli cell nucleus; $V_{VSC,T}$ (cm^3/cm^3), volume density of SC in the reference space of testes; $S_{VSC,T}$ (cm^2/cm^3), surface density of SC in the reference space of testes; SSC (μm^2), absolute surface area of an average single Sertoli cell; $S_{VSJ,T}$ (cm^2/cm^3), surface density of inter-Sertoli tight junction in the reference space of testes; ($N_{VSC,T}$), ($N_{VSN,T}$) ($10^6/cm^3$), numerical density of SC in the reference space of testes; SSJ (μm^2), absolute surface area of inter-Sertoli tight junction of a single Sertoli cell; RB, residual body; $V_{VRB,SC}$ (cm^3/cm^3), volume density of residual body in the reference space of Sertoli cell; VRB (μm^3), absolute volume of residual body in an average single Sertoli cell; PC, spermatocyte; $V_{VPC,T}$ (cm^3/cm^3), volume density of spermatocyte in the reference space of testes; rPt, round spermatid; 9PT, type 9 spermatid in stage IX; $V_{VrPt,T}$ (cm^3/cm^3), volume density of round spermatid in the reference space of testes; $V_{V9Pt,T}$ (cm^3/cm^3), volume density of type 9 spermatid in the reference space of testes; $N_{V9Pt,T}$ ($10^6/cm^3$), numerical density of type 9 spermatid in the reference space of testes.

Results

The results are summarized in tables 1, 2 and 3, and in Figures 1-7.

Hormone concentrations

Testosterone in the T group increased prominently to 7.5 times that of its counterpart in the control group ($p<0.01$); the level of its counterpart in the AT group decreased prominently compared to the level of its counterpart in the T group, until it was 0.77 times the amount of its counterpart in the control group ($p<0.05$) (Table 1).

LH in the T group decreased prominently to 0.4 times that of its counterpart in the control group ($p<0.01$), while its counterpart in the AT group markedly increased compared to its counterpart in the T group, until it was 0.38 times that of its counterpart in the control group ($p<0.05$).

FSH in the T group decreased prominently to 0.55 times that of its counterpart in the control group ($p<0.01$), whereas its counterpart in the AT group greatly increased to 2.3 times that of its counterpart in the control group ($p<0.05$). These analyses of hormones suggest that in the AT group, the levels of T, LH and FSH may show a certain tendency of recovery to their counterparts, levels in the control group, which is nearly the same situation for the morphometric analysis of the paired testes weight, $V_{VST,T}$, and the total volume of the seminiferous tubule (Table 2), [1-3].

Parameter	(C)Control	(T) Testosterone treatment for 7 days	(AT) 7 days after testosterone treatment
Testosterone	2.98 ± 0.61	22.40 ± 11.11	2.43 ± 1.49
LH	2.97 ± 0.003	0.13 ± 0.003	1.13 ± 0.19
FSH	25.73 ± 0.41	14.37 ± 2.26	32.60 ± 1.27

Table 1: Plasma concentrations (ng/ml) of testosterone, LH and FSH.

Parameter	(C)Control	(T) Testosterone treatment for 7 days	(AT) 7 days after testosterone treatment
(1) Paired testes weight (mg)	2883.7 ± 20.7	2933.3 ± 124.7	2794.0 ± 150.0
(2) $V_{VST,T}$ (cm^3/cm^3)	0.820 ± 0.004	0.739 ± 0.028	0.815 ± 0.025
(3) Total Volume of ST(cm^3)	2.271 ± 0.009	2.084 ± 0.079	2.190 ± 0.122

Table 2: Morphometric analysis in light microscopy in younger adult rats.

Parameter	(C)Control	(T) Testosterone treatment for 7 days	(AT) 7 days after testosterone treatment
(4)$V_{VSC,T}$(cm^3/cm^3)	0.1498 ± 0.013	0.1395 ± 0.013	0.1005 ± 0.013
(5)$N_{VSC,T}$($10^6/cm^3$)	21.88 ± 0.72	21.29 ± 0.04	13.27 ± 0.03
(6)VSC(μm^3)	6672.27 ± 149.49	6563.201 ± 338.918	7576.045 ± 52.93
(7) SSC(μm^2)	6962.29 ± 597.3	7182.16 ± 149.79	6434.88 ± 813.33
(8) SSJ(μm^2)	259.34 ± 53.08	541.0 ± 91.0	887.019 ± 149.202
(9)SSJ/SSC(%)	3.27 ± 1.09	3.76 ± 1.10	8.65 ± 2.30
(10) SSC(μm^2)-SSJ(μm^2)	6702.95 ± 502	6641.16 ± 564	5547.861 ± 426
(11)VSN(μm^3)	1131.33 ± 241.69	1359.11 ± 100.07	1001.67 ± 380.89
(12)VSN/VSC(%)	16.92 ± 0.31	20.75 ± 0.89	15.02 ± 5.03
(13) VVRB, SC(cm^3/cm^3)	Nearly absent	Nearly absent	0.1045 ± 0.002
(14) VRB(μm^3)	Nearly absent	Nearly absent	973.022 ± 647.71
(15) $V_{VPC,T}$(cm^3/cm^3)	0.0259 ± 0.0005	0.0664 ± 0.0048	0.1290 ± 0.0047
(16) $V_{VrPt,T}$(cm^3/cm^3)	0.1313 ± 0.0033	0.1181 ± 0.0125	0.0838 ± 0.0569
(17) $N_{V9Pt,T}$($10^6/cm^3$)	5.21 ± 0.10		89.90 ± 7.00
(18) $V_{V9Pt,T}$(cm^3/cm^3)	0.1634 ± 0.0126	0.1475 ± 0.0112	0.069 ± 0.0053
	C/C	T/C	AT/C
(19) [$V_{V9Pt,T}$/ $V_{VPC,T}$]	6.308	2.194	0.534
(20) [$V_{VrPt,T}$/ $V_{VPC,T}$]	5.069	1.779	0.650
(21) [$V_{V9Pt,T}$/ $V_{VrPt,T}$]	1.244	1.249	0.831

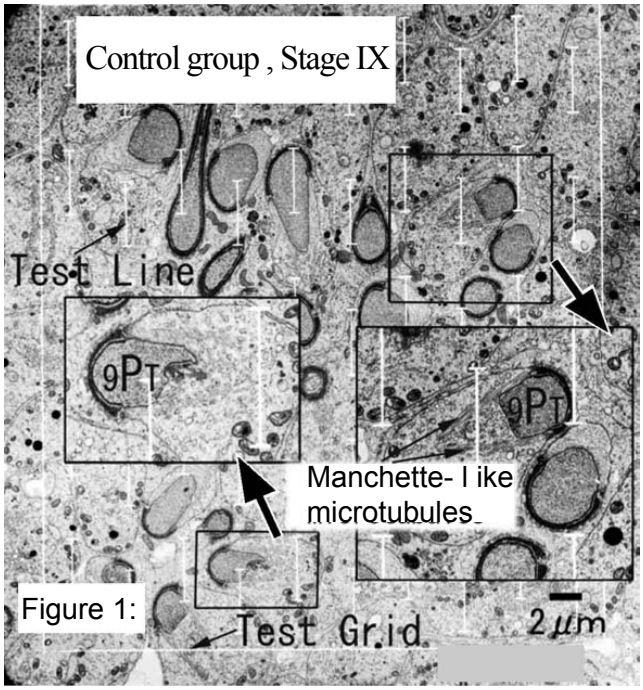
Table 3: Morphometric analysis of ultra structure of younger adult rat testes.

Morphometry of parameters for Sertoli cells [4-14]

$V_{VSC,T}$ (cm^3/cm^3) in the AT group decreased significantly ($p<0.05$) compared to its counterparts in both the control and T groups (Table 3).

VSC (μm^3) in the AT group increased significantly more than its counterparts in both the control and T groups ($p<0.05$), and the counterpart in the C group was no different from the counterpart in the T group ($p>0.05$). However, the difference between the counterparts of the C and T groups was not significant ($p>0.05$). This suggests that VSC (μm^3) in the AT group dose had not recovered to the level of its counterpart in the control group ($p<0.05$).

VSN/VSC (%) increased significantly in the T group ($p<0.05$) rather than its counterpart in the C group, whereas its counterpart in the AT group decreased significantly ($p<0.05$) rather than its counterpart in the T group. This suggests that the counterpart in the AT group shows a certain tendency to recover its counterpart level in the control group



BL: Basal Lamina of seminiferous tubular epithelium; MO: Myoid Cell; Mt(MT): Mitochondria; PC: Spermatocyte; Pt: Spermatid; 9Pt: Type 9 Spermatid; RB: Residual Body; SC; Sertoli Cell; SJ (ISC): Inter-Sertoli Cell tight junction

Figure 1: The control group (Stage IX) shows rather numerous type 9 developing spermatids. The smaller square on the right has an enlarged area with an arrow (lower right). Within this area, manchette-like microtubules are partially observed (see two small arrows). The small rectangle on the lower left has an enlarged area. Shown in the upper rectangle (superimposed arrow). Within this rectangle, the type 9 spermatids have no manchette-like microtubules. Scale bar: 2 µm.

(Table 3), [12]. This suggestion is also exemplified by the fact that the differences in counterparts between the C and AT group are smaller than those between the Control and T groups.

SSC (μm^2) did not show any significant difference between each counterpart in any of the Control, T and AT groups ($p>0.05$).

SSJ (μm^2) increased in the T group significantly ($p<0.05$) more than its counterpart in the control group, and also increased significantly ($p<0.05$) more in the AT group than its counterpart in the T group.

SSJ/SSC (%) did not show any significant difference ($p>0.05$) between its counterparts in the control and T groups, while its counterpart in the AT group increased significantly ($p<0.05$) unlike its counterpart in the T group. This suggests that the counterpart in the AT group does not recover its counterpart in the control group.

Both $V_{VRB,SC}$ ($\mu m^3/\mu m^3$) and RB (residual body) were nearly absent in Sertoli cells in both C and T groups, while the $V_{VRB,SC}$ and RB had a certain value in the cytoplasm of the Sertoli cells in the AT group. Furthermore, their location was rather predominant in the baso-lateral cytoplasm of Sertoli cells in the AT group. The location of RB and the characteristic ultrastructural feature in which mitochondria is opposed by cisternae of RER is rather in common with the Sertoli cells in the AT group.

Morphometry of parameters for spermatogenic cells

$V_{VPC,T}$ (cm^3/cm^3) in the T group increased significantly ($p<0.05$) more than their counterpart in the control group, and also increased significantly ($p<0.05$) in the AT group rather than its counterpart in the T group (Table 3), [15]. This suggests that $V_{VPC,T}$ in the AT group does not recover the level of its counterpart in the control group.

$V_{VrPt,T}$ (cm^3/cm^3) did not show any significant difference ($p>0.05$) between counterparts in all the control, T and AT groups.

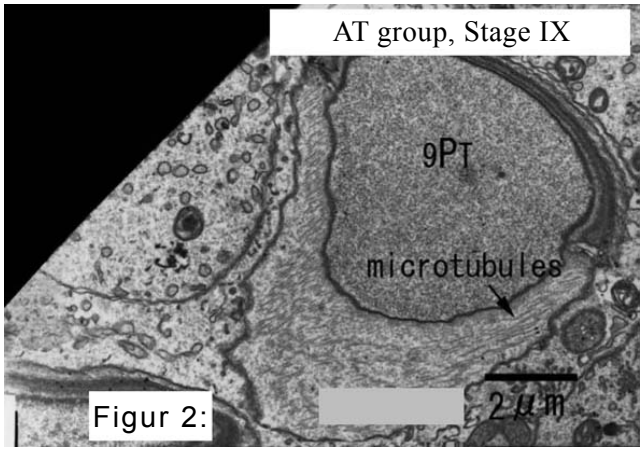
This suggests $V_{VrPt,T}$ in the AT group does not recover its counterpart in the control group.

$V_{V9Pt,T}$ (cm^3/cm^3) did not show any significant difference ($p>0.05$) in the T group compared to its counterpart in the control group, whereas its counterpart decreased significantly ($p<0.05$) in the AT group rather than in its counterpart in the T group. This suggests that the counterpart in the AT group does not recover its counterpart in the control group.

Characteristic ultrastructure in both Sertoli and spermatogenic cells

In the cytoplasm of the type 9 spermatid in the AT group, the manchette-like organized microtubules are predominant as in the control group (Figures 1,2), while the disorganized manchette-like microtubules are predominant in the cytoplasm of corresponding cells in the T group, as seen in the earlier study [1]. Furthermore, the ultrastructure corresponding to transverse sections of the ultrastructure of normal tails of spermatozoa is detected rather often in the cytoplasm of type 9 spermatids in the adluminal region of the seminiferous tubule. This suggests that normal spermatogenesis could be resumed in the AT group (Figure 3).

The morphometry (Table 3) and ultra structure (Figures 2-5,7) for Sertoli and spermatogenic cells were the focus in the AT group. $V_{VSC,T}$ (cm^3/cm^3), $N_{VSC,T}$ ($10^6/cm^3$), VSC (cm^3/cm^3), VSN/VSC (%), SSJ (μm^3), SSJ/SSC (%), $V_{VPC,T}$ (cm^3/cm^3), $V_{VRB,SC}$ (cm^3/cm^3) and VRB (μm^3) increased significantly ($p<0.05$) compared to their counterparts in the control group, while those of SSC (μm^2) decreased significantly ($p<0.05$) compared to their counterparts in the control group. The respective values for spermatogenic cells such as spermatocyte (Table



BL: Basal Lamina of seminiferous tubular epithelium; MO: Myoid Cell; Mt(MT): Mitochondria; PC: Spermatocyte; Pt: Spermatid; 9Pt: Type 9 Spermatid; RB: Residual Body; SC; Sertoli Cell; SJ (ISC): Inter-Sertoli Cell tight junction

Figure 2: AT group (Stage IX). Microtubules are being formed with a manchette-like structure. Scale bar: 2 µm.

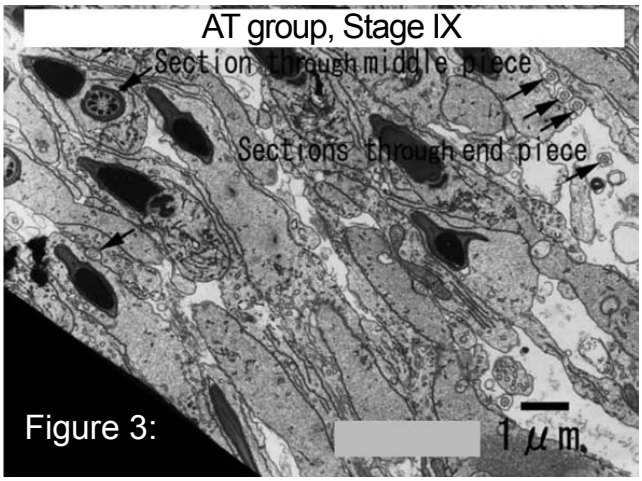


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Figure 3: AT group (Stage IX). Rather numerous structures corresponding to transverse sections of tail of spermatozoa are recognized (middle piece by arrow at top left, end piece by arrows at left middle and top right). Scale bar: 1 μm.

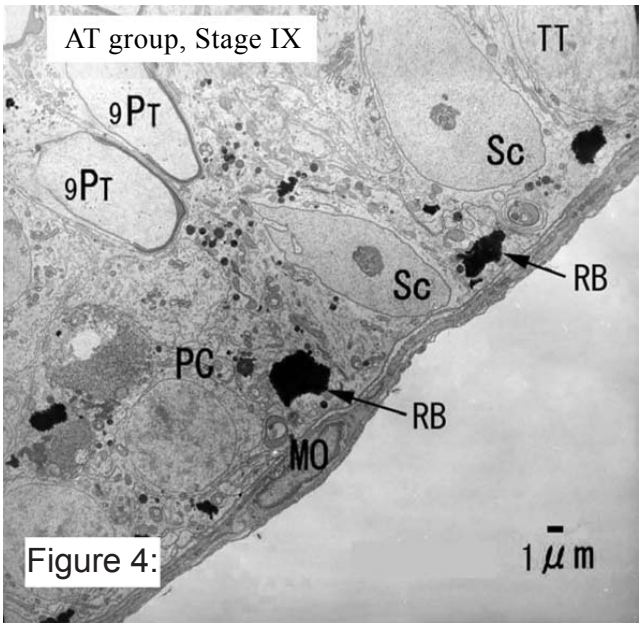

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Figure 4: AT group (Stage IX). Rather numerous and large RB (arrows) appeared in the baso-lateral cytoplasm of SC. Scale bar: 1 μm.

3), [15], round spermatid (Table 3), [16], and type 9 spermatid (Table 3), [17,18] are listed in Table 3. The volume density of spermatocytes in the reference space of testes ($V_{VPC,T}$ (cm^3/cm^3)) increased significantly ($p<0.05$), whereas both $V_{VrPt,T}$ (cm^3/cm^3) and $V_{V9Pt,T}$ (cm^3/cm^3) decreased significantly ($p<0.05$) compared to their counterpart in the control group.

Further ultra structural analysis of Sertoli cells shows that in the baso-lateral cytoplasm mitochondria with tubular cristae are closely apposed and surrounded on the outer membrane by cisternae of RER (Figures 5,6). These ultra structural features are located rather often near the inter-Sertoli tight junctions. In the AT group, RB are located rather often in common in an area of the ultra structural features of mitochondria as mentioned above (Figures 4,7), and are accompanied with a significant increase ($p<0.05$) of both SSJ (μm^2) and SSJ/SSC (%) (Table 3), [8,9] as well as with a significant ($p<0.05$) decrease of both VSN (μm^3) and VSN/VSC (%) (Table 3), [11,12].

Discussion

The recovery of spermatogenesis from the effects of testosterone treatment was studied through the morphometric analysis of testes in three different groups; control rats (C), rats immediately after 7-day testosterone treatment (T), and rats after 7-day testosterone treatment followed by 7-day non-treatment (AT). The morphometric model and the measurements and calculations by electron microscopy [1,6,7,9,10,12,13] were further modified for testicular tissue focusing on spermatogenesis in stage IX. For brevity, the abbreviations for morphometric terms are used. These abbreviations are explained in the Abbreviations for Morphometric Terms point.

The present results (Tables 1-3) are in good agreement with those in the previous study [1], and all the corresponding values match reasonably well those in earlier publications [14-19] as with $V_{VSC,T}$,

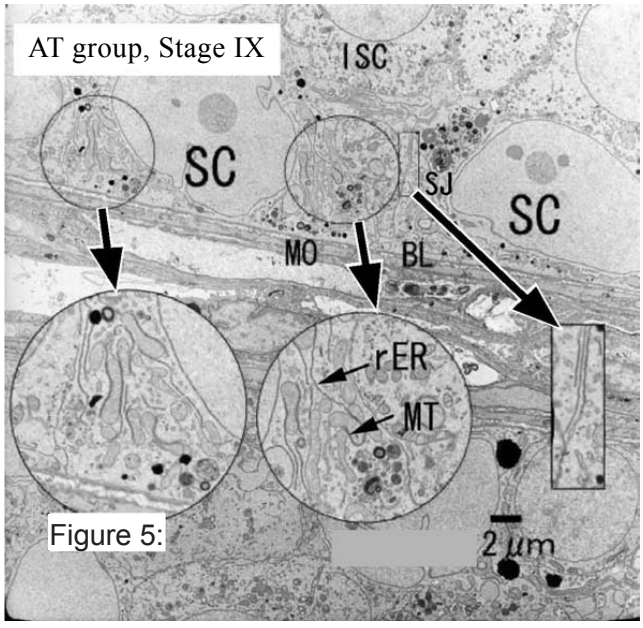

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Figure 5: AT group (Stage IX). Cytoplasms within upper two small circles are enlarged and superimposed (arrows) within lower two larger circles. They show close apposition of MT and RER in the baso-lateral area of two neighboring Sertoli cells. Upper small rectangular area is enlarged and superimposed (arrow) as lower rectangular area at right. This shows the ISC (SJ) between baso-lateral cell surfaces of two neighboring Sertoli cells. Scale bar: 2 μm.

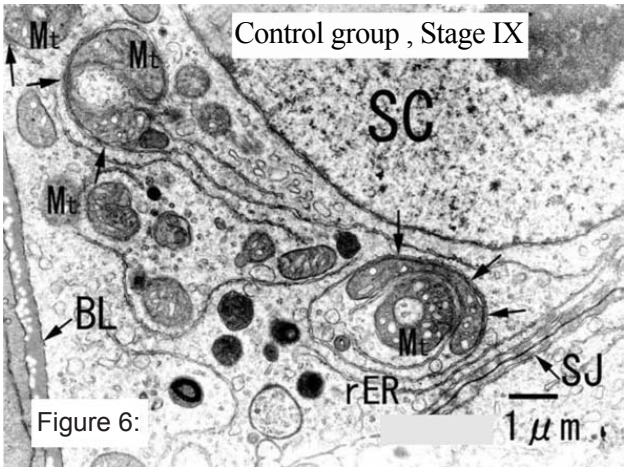

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Figure 6: Control group (Stage IX). Close apposition of RER to outer membrane of Mt (three arrows at top left and three arrows at lower right) are noted, and SJ (ISC) is seen near aforementioned ultrastructure. Scale bar: 1 μm.

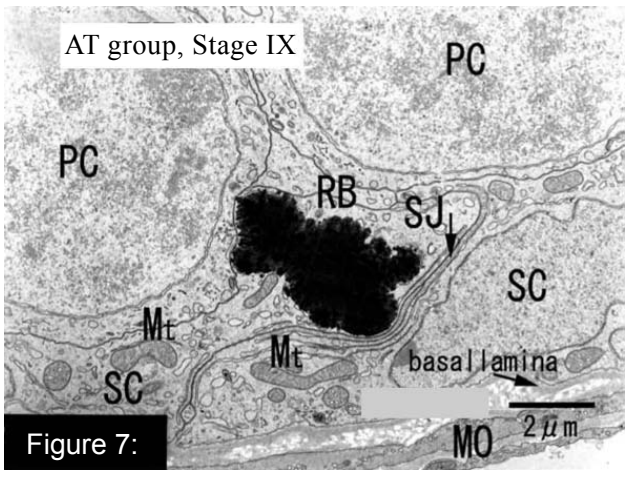

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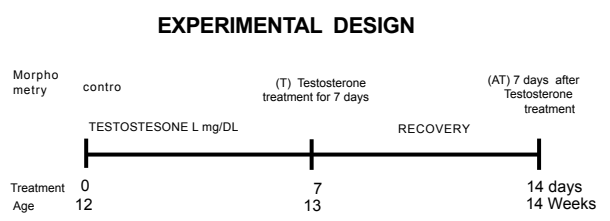
Figure 7: AT group (Stage IX). Baso-lateral cytoplasm in Sertoli cell is occupied by a rather large RB. SJ is indicated by arrow. Scale bar: 2 μm.



BL: Basal Lamina of seminiferous tubular epithelium; MO: Myoid Cell; Mt(MT): Mitochondria; PC: Spermatocyte; Pt: Spermatid; 9Pt: Type 9 Spermatid; RB: Residual Body; SC; Sertoli Cell; SJ (ISC): Inter-Sertoli Cell tight junction

Figure 8: Experimental Design.

$N_{VSN,T}$, VSC & SSC, SSJ. In the present AT group several parameters such as VVSC,T, SSJ, VSN/VSC, $V_{VPC,T}$, $V_{VrPt,T}$ and $V_{V9Pt,T}$, (Table 3), [4,8,12,15,16] showed significant morphometric alterations which were not expected from the absence of respective receptors for both T and FSH in the Sertoli cell nucleus, as in the T group in the previous study [1]. These are not explained only by results [19-23] in which Sertoli cells and spermatogenic cells show strong interactions only via hormones such as testosterone, LH and FSH. In stage IX, both of the minimal androgen receptors in Sertoli cells [2] and the significantly low FSH are recognized together with minimal mRNA of FSH receptors in Sertoli cells [3,4]. The present results therefore suggest a non-hormonal regulatory mechanism for the detected changes. These results obtained from morphometry and ultra structure may suggest that the regulatory action of Sertoli cells towards spermatogenesis involves systematic structural changes in the cell nucleus, mitochondria-RER complex in the basolateral cytoplasm, inter-Sertoli tight junctions, and finally the SSJ/SSC (%), [SSC (μm^2) - SSJ (μm^2)] (Table 3), [9,10]. This view is supported by the significant increase ($p<0.05$) in $V_{VPC,T}$ in the AT group rather than its counterpart in the control group, and also by the following significant decrease ($p<0.05$) of both $V_{VrPt,T}$ and V_{V9PtT} in the AT group rather than its counterpart in the control group. These decreases may be due to insufficient development from PC to rPt, followed by the insufficient development of 9Pt. On the other hand, this is associated with a significant decrease in SSC (μm^3) - SSJ (μm^3) ($p<0.05$) as well as a significant increase in SSJ/SSC (%) ($p<0.05$) in the AT group, rather than their counterparts in the control group. This significant decrease in the free surface of SSC may suggest a certain shortage of information necessary for ordinary spermatogenesis from Sertoli cells to contact spermatocytes [21,23].

The recovery of testes in rats after irradiation can be stimulated by GnRH antagonist treatment. The addition of androgens in these conditions inhibited recovery [24], whereas estradiol was not inhibitory.

Conclusions

The present observations strongly suggest that significant regulatory factors in spermatogenesis remain to be discovered, as in earlier observations [1].

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Summary Sentence

The present study was conducted to determine the morphometry of rat Sertoli and spermatogenic cells in stage IX immediately after 7-day testosterone treatment in one group and with the same treatment in another group followed by 7-day non-treatment. The results suggested that a still unknown number of regulatory factors are apparently involved in the developmental interactions between Sertoli and spermatogenic cells.

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References

1. Ichihara I, Pelliniemi LJ (2007) Morphometric and ultrastructural analysis of stage-specific effects of Sertoli and spermatogenic cells seen after short-term testosterone treatment in young adult rat testes. Ann Anat 189: 520-532.
2. Vornberger W, Prins G, Musto NA, Suarez-Quian CA (1994) Androgen receptor distribution in rat testis: new implications for androgen regulation of spermatogenesis. Endocrinology 134: 2307-2316.
3. Parvinen M, Marana R, Robertson DM, Hansson V, Ritzén EM (1980) Functional cycle of rat Sertoli cells: differential binding and action of follicle-stimulating hormone at various stages of the spermatogenic cycle. In: Steinberger A, Steinberger E. (Eds.), Testicular development, Structure, and Function. Raven Press, New York, NY, 425-432.
4. Heckert LL, Griswold MD (1991) Expression of follicle-stimulating hormone receptor mRNA in rat testes and Sertoli cells. Mol Endocrinol 5: 670-677.
5. Nieschlag E, Loriaux DL (1972) Radioimmunoassay for plasma testosterone. Z Klin Chem Klin Biochem 10: 164-168.
6. Ichihara I, Kawamura H, Pelliniemi LJ (1993) Ultrastructure and morphometry of testicular Leydig cells and the interstitial components correlated with testosterone in aging rats. Cell Tissue Res 271: 241-255.
7. Ichihara I, Kawamura H, Nakano T, Pelliniemi LJ (2001) Ultrastructural, morphometric, and hormonal analysis of the effects of testosterone treatment on Leydig cells and other interstitial cells in young adult rats. Ann Anat 183: 413-426.
8. Hess RA (1990) Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. Biol Reprod 43: 525-542.
9. Weibel ER (1979) Practical methods for biological morphometry. Stereological methods: Theoretical foundations, Academic Press, London.
10. Weibel ER, Paumgartner D (1978) Integrated stereological and biochemical studies on hepatocytic membranes. II. Correction of section thickness effect on volume and surface density estimates. J Cell Biol 77: 584-597.
11. Ichihara I, Kawai N, Heilbronner R, Rohr H (1985) Stereologic and fine-structural studies of prostatic acinar basal cells in the dog. Cell Tissue Res 242: 519-525.
12. Weibel ER (1969) Stereological principles for morphometry in electron microscopic cytology. Int Rev Cytol 26: 235-302.
13. Weibel ER, Kistler GS, Scherle WF (1966) Practical stereological methods for morphometric cytology. J Cell Biol 30: 23-38.
14. Bugge HP, Plöen L (1986) Changes in the volume of Sertoli cells during the cycle of the seminiferous epithelium in the rat. J Reprod Fertil 76: 39-42
15. Kerr JB (1988) A light microscopic and morphometric analysis of the Sertoli cell during the spermatogenic cycle of the rat. Anat Embryol 177: 341-348.
16. Russell LD, Ren HP, Sinha-Hikim I, Schulze W, Sinha-Hikim AP (1990) A comparative study in twelve mammalian species of volume densities, volumes, numerical densities of selected Testis component, emphasizing those related to the Sertoli cell. Am J Anat 188: 21-30.
17. Ghosh S, Bartke A, Grasso P, Reichert LE, Russell LD (1992) Structural manifestations of the rat Sertoli cell to hypophysectomy: a correlative morphometric and endocrine study. Endocrinology 131: 485-497.
18. Weber JE, Russel LD, Wong V, Peterson RN (1983) Three dimensional reconstruction of a rat stage V Sertoli cell: II. Morphometry of Sertoli-Sertoli and Sertoli-germ cell relationships. Am J Anat 167:163-179.
19. Hansson V, Weddington SC, McLean WS, Smith AA, Nayfeh SN, et al. (1975) Regulation of seminiferous tubular function by FSH and androgen. J Reprod Fertil 44: 363-375.
20. Salhanick AI, Terner C (1979) Androgen synthesis in absences of Leydig and Sertoli cells in a germ cell fraction from rat seminiferous tubules. Biol Reprod 21: 293-300.
21. Onoda M, Djakiew D, Papadopoulos V (1991) Pachytene spermatocytes regulate the secretion of Sertoli cell protein(s) which stimulate Leydig cell steroidgenesis. Mol Cell Endocrinol 77: 207-216.
22. El Chami N, Ikhlef F, Kaszas K, Yakoub S, Tabone E, et al. (2005) Androgen-dependent apoptosis in male germ cells is regulated through the proto-oncoprotein Cbl. J Cell Biol 171: 651-661.
23. O'Shaughnessy PJ, Verhoeven G, De Gendt K, Monteiro A, Abel MH (2010) Direct action through the Sertoli cells is essential for androgen stimulation of spermatogenesis. Endocrinology 151: 2343-2348.
24. Shetty G, Wilson G, Hardy MP, Niu E, Huhtaniemi I, et al. (2002) Inhibition of recovery of spermatogenesis in irradiated rats by different androgens. Endocrinology 143: 3385-3396.