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BIOCHEMICAL AND HISTOPATHOLOGICAL ALTERATIONS IN A UNILATERAL CRYPTORCHIDISM MODEL AFTER hCG TREATMENT

TEK TARAFLI İNMEMİŞ TESTİS MODELİNDE hCG TEDAVİSİ SONRASI BİYOKİMYASAL VE HİSTOPATOLOJİK DEĞİŞİKLİKLER

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ABSTRACT

Purpose: To determine the effects of Human Chorionic Gonadotropin (hCG) treatment in unilateral undescended testis model by determining serum testosterone and antisperm antibody (ASA) levels and using stereological methods in prepubertal rats.

Methods: A total of 40 prepubertal (30-day old) male Sprague-Dawley rats were separated into Sham, Sham+ hCG treatment, Undescended testis and Undescended testis+ hCG treatment groups. The rats were sacrificed following a 30-day undescended testis duration and 30-day hCG treatment duration. Blood samples were obtained to determine the ASA and testosterone levels. The testes were prepared following routine histological procedures. The seminiferous tubule (parenchyma) and insterstitium (stroma) (P/S) ratios were calculated using stereological methods.

Results: The testosterone levels were 689.08±96.08 ng/dl in the Sham group, 1062.32±78.5 ng/dl in the Sham+hCG group, 176.09±23.35 ng/dl in the Undescended testis group and 328.75±43.49 ng/dl in the Undescended testis+hCG group. ASA results were 2.94±0.56 U/ml in the Sham group, 5.45±1.58 U/ml in the Sham+hCG group, 20.87±4.76 U/ml in the Undescended testis group, and 22.12±6.99 U/ml in the Undescended testis+hCG group. A statistically significant difference was detected between the Undescended testis and Sham groups. The comparison of the parenchyma/stroma (P/S) ratios revealed statistically significant difference between Sham groups and Undescended testis groups ($p<0.05$). There was also statistically significant difference in P/S ratios of Undescended testis and Undescended testis+HCG treatment groups ($p<0.05$).

Conclusion: In this study, it was found that hCG changed the P/S ratio in favor of the parenchyma. The higher level of ASA in groups with an undescended testis indicated that ASA production could be higher in testes that had undergone more handling. Although hCG treatment improved testosterone levels, this treatment did not decrease ASA production in unilateral cryptorchidism. Our findings lead to the conclusion that hormonal treatment effects morphological changes and testosterone levels but could not eliminate elevated ASA production. Further experimental studies can be performed in order to elucidate the mechanisms that effect the other testis in unilateral cryptorchidism.

Key words: ASA, hCG, stereology, testosterone, undescended testis

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ÖZET:

Amaç: Prepubertal sıçan kullanılarak oluşturulan unilateral inmemiş testis modelinde Human Koriyonik Gonadotropin (hCG) tedavisinin etkilerinin serum testosteron ve antisperm antikor (ASA) düzeyleri saptanarak ve stereolojik metodlar kullanılarak değerlendirilmesi.

Yöntem ve Gereçler: Toplam 40 adet prepubertal (30 günlük) erkek Spraque-Dawley sıçan Sham, Sham+ hCG tedavisi, İnmemiş testis ve İnmemiş testis+ hCG tedavisi gruplarına ayrıldı. Sıçanlar 30 günlük inmemiş testis ve 30 günlük hCG tedavisi sonrasında sakrifiye edildi. Serum ASA ve testosteron düzeylerini saptamak için kan örnekleri alındı. Testis dokuları rutin histopatolojik işlemlere alındı. Seminifer tübül (parankim) ve interstisiyel alan (stroma) (P/S) oranları stereolojik yöntemler kullanılarak hesaplandı.

Bulgular: Testosteron düzeyleri Sham grubunda 689.08 ± 96.08 ng/dl , Sham + hCG grubunda 1062. 32 ± 78.5 ng/dl , İnmemiş testis grubunda 176.09 ± 23.35 ng/dl ve İnmemiş testis+ hCG grubunda 328.75 ± 43.49 ng/dl ölçüldü. ASA düzeyleri Sham grubunda 2.94 ± 0.56 U/ml , Sham + hCG grubunda 5.45 ± 1.58 U/ml, İnmemiş testis grubunda 20.87 ± 4.76 U/ml ve İnmemiş testis+ hCG grubunda 22.12 ± 6.99 U/ml ölçüldü. Testosteron ve ASA düzeyleri açısından Sham grupları ile İnmemiş testis grupları arasında fark saptandı. Parankim/stroma (P/S) oranları karşılaştırıldığında Sham grupları ile İnmemiş testis grupları arasında istatistiksel anlamlı fark saptandı ($p<0.05$). Ayrıca, P/S oranları açısından değerlendirildiğinde İnmemiş testis ve İnmemiş testis+ hCG grubunun arasında da istatistiksel fark saptandı.

Sonuç: Çalışmada hCG tedavisinin P/S oranını parankim lehine çevirdiği bulunmuştur. İnmemiş testis gruplarında ASA düzeylerinin daha yüksek bulunması, çok işleme tabi tutulan testislerde ASA üretiminin artabileceğini düşündürmüştür. hCG tedavisi inmemiş testis grubunda testosteron düzeyini arttırmasına rağmen ASA düzeyini azaltamamıştır. Bulgularımız sonucunda inmemiş testis grubunda hormonal tedavinin morfolojik değişiklikleri ve testosteron düzeylerini etkilediği ancak artmış ASA düzeyini indiremediği saptanmıştır, bu konuya açıklık getirmek için planlanan deneysel çalışmalar unilateral inmemiş testiste karşı testisi de etkileyen patolojik mekanizmaların açıklanmasında yol gösterici olacaktır.

Anahtar Kelimeler: ASA, hCG, stereoloji, testosteron, inmemiş testis

INTRODUCTION

Undescended testis is the most common disorder of male sexual differentiation (1). The growth and development of testis and related structures depends on adequate chorionic gonadotropin and androgen levels during pregnancy (2). The number of Leydig cells is decreased in most undescended testis cases, together with the germ cells in some (3).

Damage has been found in both the undescended testis and contralateral testis in experimental undescended testis models (4,5). Hormonal treatment for undescended testis has entered clinical use in the last few years. Treatment with gonadotropins in the early stage for this condition stimulates germ cell maturation and increases the fertility potential following surgery (6,7). Treatment with luteinizing hormone secreting hormone and hCG have been clearly shown to promote descent of the testis into the scrotum (8). However, some studies report an increase in the apoptosis rate of germ cells and decreased testicular volume on long-term follow-up in undescended testis cases treated with hCG (9).

Investigators such as Landsteiner and Metchnikoff have shown the immunogenicity of spermatozoa almost a century ago (10, 11). Studies have been performed on the future effect on fertility of antisperm antibodies. One study has found no evidence of any role of antisperm antibody (ASA) in the spermatogenesis damage in unilateral undescended testis cases while others have shown the presence of ASA in 28-52% of the cases (12-14).

The aim of this study was to evaluate the changes with hCG (Pregnyl) in a unilateral undescended testis

model with histopathological methods and the biochemical measurement of ASA and serum testosterone levels.

MATERIALS AND METHODS

We used a total of 40 prepubertal (30-day old*) Sprague-Dawley type male rats in our study. The Ethics Review Board of our institute has approved the study protocol. The rats were obtained from the animal laboratory of Suleyman Demirel University Medical School. They were kept in separate cages with heat and light control and provided adequate food and water according to the U.S. National Institutes of Health laboratory animal use and care guidelines (NIH publication No. 85-23, revised 1985).

Surgical model:

The surgical procedures were performed under ketamine hydrochloride (50 mg/ml) anesthesia (50 mg/kg, im) using sterile technique. The abdomen was shaved and prepared with 10% povidone-iodine solution and a midline incision performed to access the left testicle. Fourty rats divided in to four groups and each group contained ten rats.

Group I (Sham): The left testis was removed from the abdomen and then replaced. The rats in this group were sacrificed 60 days after the first operation and the testicles removed.

Group II (Sham + hCG treatment): The left testicle was removed from the abdomen and then replaced. hCG (Pregnyl, Organon) treatment at a dosage of 100 IU was started 30 days after the first surgery using the treatmentonce a week for 4 weeks (15). The rats were sacrificed under ketamine anesthesia 60 days after the first operation and the testicles were removed.

Group III (Undescended testis): The gubernaculum testis was cut and the left testis fixed to the left abdominal wall using 6/0 nylon sutures to create an undescended testis model. Rats in this group were sacrificed under ketamine anesthesia 60 days after the first surgery and the testicles were removed.

Group IV (Undescended testis + hCG treatment): The gubernaculum testis was cut and the left testis fixed to the left abdominal wall using 6/0 nylon sutures to create an undescended testis model. hCG (Pregnyl, Organon) treatment at a dose of 100 IU was started 30 days after the first surgery using the treatment once a week for 4 weeks. The rats were sacrificed under ketamine anesthesia 60 days after the first operation and the testicles were removed.

Serum samples:

Blood samples were kept at least 3 hours at 4 0C for coagulation. Serum samples were obtained by centrifuging at 2000 rpm for 10 minutes and put in glass tubes with plastic caps, labeled and kept at -780 C to study testosterone and ASA.

Testosterone Determination:

The quantitative measurement of testosterone in rat serum was performed with the Coat A Count total testosterone kit (Diagnostic Products Corporation, LA, U.S.A.) using Radioimmunoassay (RIA) methods according to the recommendations of the manufacturer. The results were expressed as nanogram/mililiter (ng/ml).

Antisperm antibody test (ASA):

ASA levels in the rat serum were measured with the Bioserv Diagnostics Sperm-Antibody kit (Bioserv-Diagnostics, Rostck, Germany) using an enzyme-linked immunoassay (ELISA) method.

Morphometric measurements:

Once the testes were removed, their weight was measured with a sensitive electronic scale. The width, height and thickness of each testis was measured with a caliper. The testes were then fixated in 10% formalin and embedded in paraffin blocks after undergoing the alcohol series and xylol procedures. Serial sections 50 µm thick were then obtained in the transverse plane and stained with Hematoxylin - Eosin (H-E). The Cavalier principle was used to obtain 15-20 sections from a single sample series using the “systematic random sampling” method. A light microscope (Olympus BX50) and counting plates placed on the sections were used to determine the parenchyma and stroma ratio. Points corresponded with seminifer-

ous tubules were evaluated as parenchyma tissue while all areas other then seminiferous tubules were accepted as stroma. The volume density of each component was expressed as a percentage of the testis volume.

Statistical evaluation:

The SPSS 10.0 statistical software (SPSS Inc. USA) was used for all statistical analysis. The Kruskal-Wallis test was used to determine the presence of any statistical difference between the groups for testis weight, testosterone, ASA levels, right and left testis parenchyma and stroma ratios, and any changes with hCG treatment. A p value <0.05 was considered as statistically significant.

RESULTS

The testosterone levels of the groups were as follows: Sham group, 689.08 ± 96.08 ng/dl; Sham + hCG group, 1062. 32 ± 78.5 ng/dl; Undescended testis group, 176.09 ± 23.35 ng/dl; and Undescended testis + hCG group, 328.75 ± 43.49 ng/dl.

ASA levels were as follows: Sham group, 2.94 ± 0.56 U/ml; Sham + hCG group, 5.45 ± 1.58 U/ml; Undescended testis group, 20.87 ± 4.76 U/ml; Undescended testis + hCG group, 22.12 ± 6.99 U/ml.

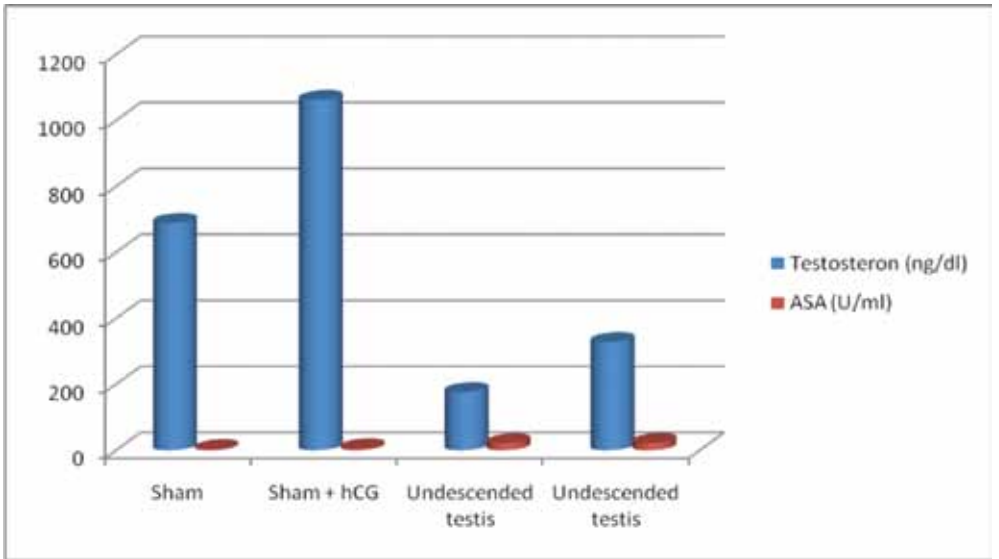
There was a statistically significant difference between the Undescended testis and Sham groups regarding testosterone and ASA levels ($p<0.05$).

Also, a significant difference was detected between the Sham and Sham + hCG groups regarding both testosterone and ASA levels ($p<0.05$).

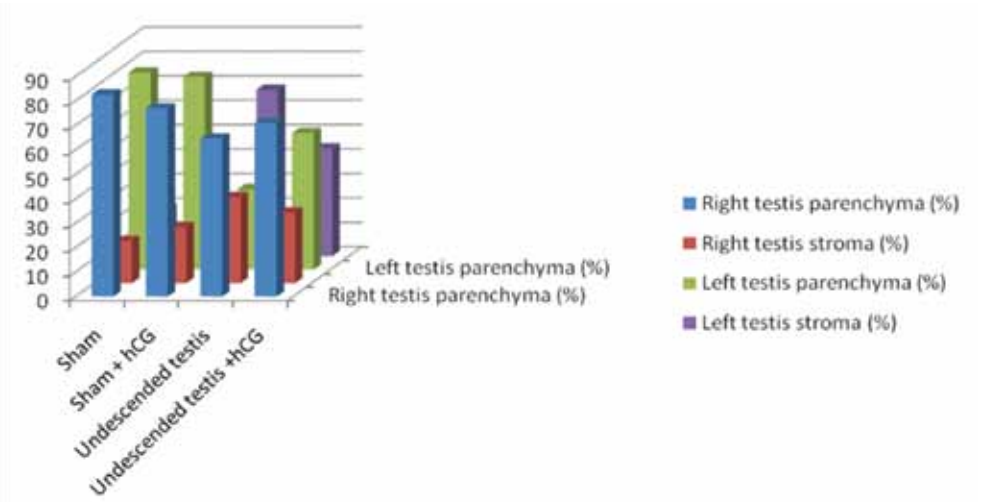
Although, there was a significant difference between the Undescended testis and Undescended testis + hCG groups regarding testosterone levels ($p<0.05$), no significant difference was found between these groups regarding ASA levels ($p>0.05$).

P/S ratios, testis weights, testosterone and ASA levels of all groups are presented in graphics (Graphic 1 and 2).

The comparison of P/S ratios of the Sham group with the Sham + hCG group showed no statistically significant difference ($p>0.05$) (Figure 1 and 2). When the P/S ratio of the right and left testis was compared in the Undescended testis group, a statistically significant difference was found ($p<0.05$) (Figure 3 and 4). When the



Graphic 1: The testosterone and ASA levels of all groups



Graphic 2: Comparison of P/S ratios of all groups

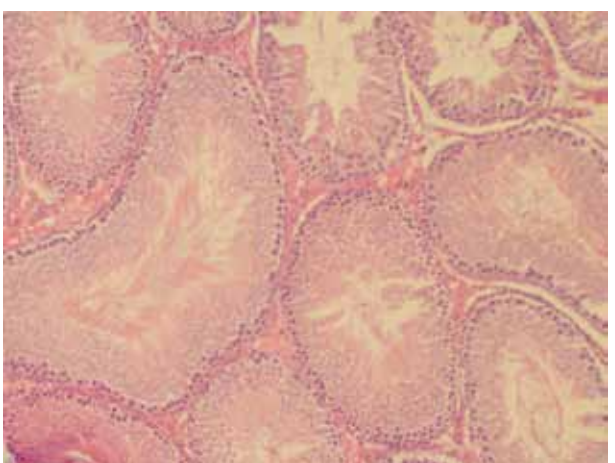


Figure 1: Testis section of Sham group

P/S ratio of this group is in normal range because parenchyma occupies most of the area. Normal morphology of the seminiferous tubules and germ cells are seen (H-E,X20).

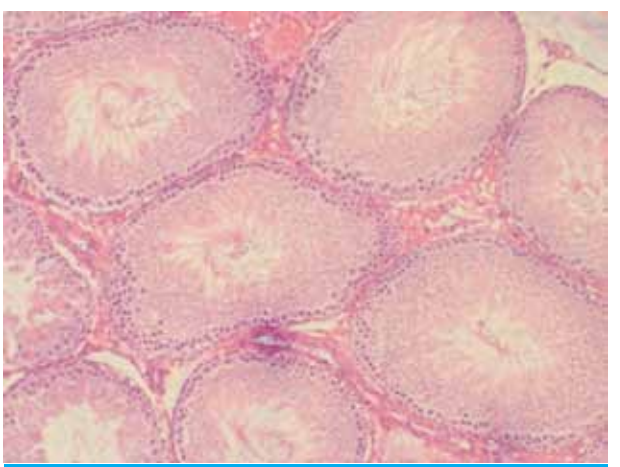


Figure 2: Testis section of Sham + hCG group

P/S ratio of this group isn't statistically different than the Sham group. Normal morphology of the seminiferous tubules and germ cells are also seen (H-E,X20).

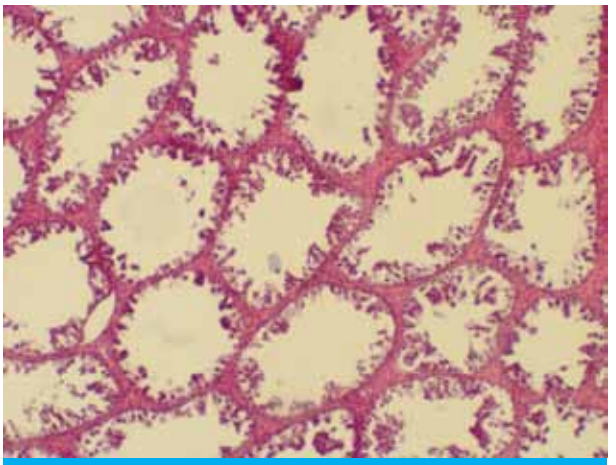


Figure 3: Left testis section of Undescended testis group

P/S ratio of this group is decreased because stroma occupies most of the area. Morphology of the seminiferous tubules and germ cells are significantly different than normal (H-E, X20).

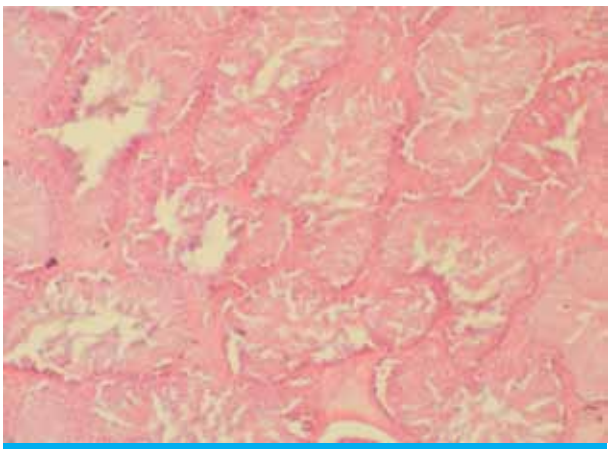


Figure 4: Right testis section of Undescended testis group

P/S ratio of this group is statistically different than Left testis of Undescended group. Morphology of the seminiferous tubules and germ cells are slightly different than normal (H-E, X20)

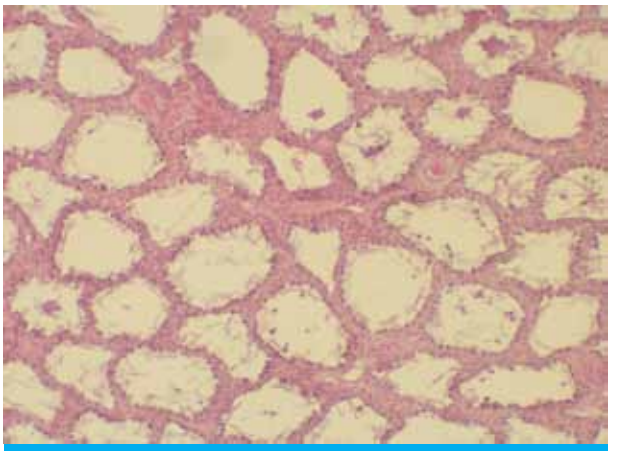


Figure 5: Left testis section of Undescended testis + hCG group

P/S ratio of this group is also decreased as stroma occupies most of the area. Morphology of the seminiferous tubules and germ cells are still significantly different than normal (H-E, X20).

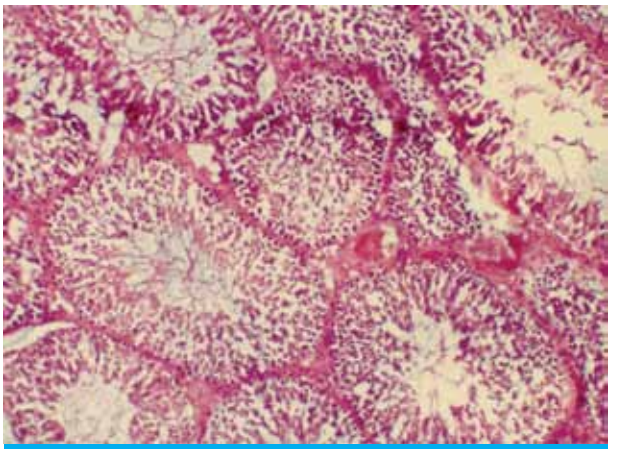


Figure 6: Right testis section of Undescended testis + hCG group

P/S ratio of this group is statistically different than Left testis of Undescended group +hCG group. Morphology of the seminiferous tubules and germ cells aren't so different than normal (H-E, X20).

P/S ratio of the right and left testis was compared in the Undescended testis + hCG group, there was also a statistically significant difference between right and left testis ($p<0.05$) (Figure 5 and 6).

A statistically significant difference was detected again when the P/S ratios of the right and left testis in the Undescended testis group was compared with the same ratios in the Undescended testis + hCG group ($p<0.05$). The right testis P/S ratio in the Undescended testis group was significantly different than the right testis P/S ratio in the Undescended testis + hCG group ($p<0.05$). When the right testis (contralateral) P/S ratio in the Sham group was compared with the right testis P/S ratio in the Undescended testis + hCG group ($p<0.05$), there was also a statistically significant difference.

DISCUSSION AND CONCLUSION

The experimental undescended testis model results in a functional and morphological changes in all the main cell types of the testis. Germ cells are degenerated and Sertoli cells show functional and morphological changes. These changes are explained with the thermal stress the undescended testis is exposed to. The mechanism of the thermal stress includes direct and indirect effects on germ cell apoptosis, the development of heat shock proteins, defective germ cell maturation, reactive oxygen products (ROS), and Sertoli cell damage (16-18).

There are many studies reporting damage in the contralateral testis in unilateral undescended testis cases (4,5). It was also reported that some unknown factors related to the highly impaired unilateral undescended testis may inhibit the function of the contralateral unaffected testis (19). Previous studies have reported these changes as an increase or decrease with no numerical data. In this study, we evaluated data to identify the quantitative morphological changes in the testis that occur in experimental cryptoorchidism model in prepubertal rats.

The present study demonstrated that degeneration of germ cells resulted in a reduction in the weight and P/S ratio of experimental undescended testis in prepubertal rats. Although hCG treatment led to a relative increase in P/S ratio and serum testosterone levels, the treatment would not be able to decrease ASA levels in damaged testis groups. The reduction of germ cells lead to a weight loss in undescended testis groups, whereas P/S ratio also decreased markedly due to the reduction in tubule volume. This observation emphasizes that in all morphological studies it is important to use stereological methods to evoid erroneous conclusions.

Although there was no significant difference between P/S ratios of right and left testis in Sham groups,there was statistically significant difference between the P/S ratios of the right and left testis in the Undescended testis group. There was also a significant difference between the P/S ratios of the right and left testis in the Undescended testis + hCG group. A significant difference was also found when the P/S ratios of the right and left testis in the Undescended testis group were compared with the same ratios in the Undescended testis + hCG group.

Unilateral undescended testis model in dogs has demonstrated that the spermatogenic and endocrine functions of the contralateral testis are suppressed by the undescended testis (20). The marked decrease in the volume of the undescended testis was thought to be due to a decrease in all germ cells other than spermatogonia and the slight reduction in volume and seminiferous tubule diameter in the contralateral testis was thought to be due to a mild decrease in germ cells. A previous study has reported apoptosis in the undescended testis in unilateral undescended testis cases with more marked apoptosis being seen in the contralateral testis (21).

We found a significant difference between the right and left testis weight in Undescended testis groups. This difference was more significant when the right and left testis of Undescended testis groups were compared with those of the Sham groups.

Undescended testis should be treated to prevent the risk of inguinal hernia, testis torsion and malignancy and

future infertility. Many investigators recommend that this treatment be undertaken before irreversible changes develop in testicular morphology.

ASA development in males is due to the sequestration of antigens on germ cells as a result of the blood-testis barrier. New antigens are expressed on the spermatocytes and spermatids that develop during spermatogenesis. ASA develop when these antigens come into contact with immunocompetent cells. Developmental abnormalities of the blood-testis barrier, traumatic lesions or unilateral focal crypt obstructions lead to the development of ASA. Some articles report the presence of active local immunoregulatory mechanisms within the testis (22). We found significantly high ASA levels in our Undescended testis and Undescended testis + hCG groups compared to the Sham and Sham + hCG groups. Antisperm antibodies cause immune infertility, a type of autoimmune disease in males.

There was a marked difference in testosterone levels between the Undescended testis group, Undescended testis + hCG group, Sham group and Sham + hCG group in our study. Similarly, the ASA level was markedly high in groups with undescended testis when the Undescended testis group, Undescended testis + hCG group, Sham group and Sham + hCG group were compared. There was no statistically significant difference between the Undescended testis group and the Undescended testis + hCG groups for ASA levels. It was also interesting to find a statistically significant difference for ASA levels between the Sham group and the Sham + hCG group.

Taking all the data into account, it is possible to say that the contralateral testis is also damaged in undescended testis cases and that this damage can be reversed somewhat but not completely with hCG treatment. We also found ASA development to be more marked in normal testis tissue following hCG treatment. ASA levels were significantly high in the Undescended testis and Undescended testis + hCG groups where the testis had undergone more handling to create an undescended testis model.

hCG treatment shifts the P/S ratio in favor of parenchyma as regards the resolution of the damage in unilateral undescended testis and the contralateral testis. There are favorable changes in the testosterone level with hCG treatment but normal testicular testosterone levels are not achieved. hCG treatment was found not to have any favourable effect on ASA production in damaged testis tissue while it increased ASA production in normal testis tissue. The decision to start hCG treatment or the best time to perform surgery for undescended testis should therefore be carefully considered.

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