

UNIVERSIDADE ESTADUAL DE CAMPINAS

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**ANÁLISE HISTOLÓGICA E ESTEREOLÓGICA DO EPIDÍDIMO E CÉLULAS
DE LEYDIG EM CAMUNDONGOS SUBMETIDOS AO CRIPTORQUIDISMO
EXPERIMENTAL E ORQUIDOPEXIA.**

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Celular e Estrutural, na área de Biologia
Celular.

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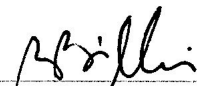
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Dedico este trabalho

À minha mãe, *Carmen*,
por todo amor, ternura, apoio,
por todo cuidado, por todo sorriso
e por mostrar que através do
trabalho que conseguimos alcançar
nossos objetivos...

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por todo sacrifício, dedicação,
por fazer parte de minha vida
e ser meu amigo...

Ào meu irmão, *Frick*,
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por todo carinho, companheirismo
e por ser um menino tão perseverante
em sua vida...

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atenção, por todo carinho e
por ser um menino tão especial
(apesar de mercear puxões de orelha)...

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por todo carinho e respeito,
por todo exemplo de vida que
reflete o grande homem que é.

Onde não falta vontade existe sempre um caminho...

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Agradeço a Deus,
por ter me conduzido a todos os caminhos de minha vida
e por ter me ajudado a me encontrar
nos momentos menos afortunados.

ERGA-SE...

Sabe aquele momento que a gente pensa que chegou ao limite das próprias forças e que não vai mais conseguir avançar? Quando não contamos as lágrimas (e nem devemos!) e tudo parece um grande vazio...

Esse momento que, não importa a nossa idade, pensamos que já é o fim... e um desânimo enorme toma conta da gente...

Esse momento, ao contrário do que parece, é justamente o ponto de partida!

Se chegarmos a um estado em que não avançamos mais, é que devemos provavelmente tomar uma outra direção.

Quando chegamos a esse ponto de tal insatisfação é sinal de que alguma coisa deve ser feita.

Não espere que os outros construam pra você, planeje e faça! Você é responsável pelos próprios sonhos e pela realização destes. Nas obras da vida não precisamos de arquitetos para planejar por nós. Com um pouco de imaginação e um pouco de boa vontade podemos reconstruir sozinhos a casa que vamos morar e o futuro que nos ofereçemos.

É humano se sentir fragilizado às vezes e mesmo necessário para que tenhamos consciência que não somos infalíveis, não somos super-heróis, mas seria desumano parar por aí. É injusto. Para os outros, mas principalmente para consigo mesmo. Recomeçar é a palavra! Recomeçar cada vez, a cada queda, a cada fim de uma estrada! Insistir!...

Se alguém te feriu, cure-se!
Se te derrubaram, levante-se!

Se te odeiam, ame!

Erga-se! Erga a cabeça!

Olhando pra baixo só podemos ver os próprios pés. É preciso olhar pra frente.

Plante uma árvore, faça um gesto gentil, tenha uma atitude positiva. É sempre possível fazer alguma coisa!

Não culpe os outros pelas próprias decepções, pelos próprios fracassos. Se somos nossos próprios donos para as nossas vitórias, por que não seríamos para as nossas derrotas?

Onde errou, não erre mais! Onde caiu, não caia mais! Se você já passou por determinado caminho, deve ter aprendido a evitar certas armadilhas.

Então, siga!

Não se esqueça de uma grande promessa feita na Bíblia:

"Esforça-te e eu te ajudarei."

Dê o primeiro passo... depois caminhe!!!

Tenho certeza que a felicidade não mora ao seu lado, nem à sua frente, ela está
junto de você!

Descubra-se, faça-se feliz!

Autora: Leticia Thompson

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A maior recompensa do nosso trabalho não é o que nos pagam por ele,
mas aquilo em que ele nos transforma.
(John Ruskin)

RESUMO

O epidídimo de mamíferos, através da secreção de proteínas andrógeno-dependentes, é responsável pela maturação dos espermatozóides visando o processo de fecundação, alterando-os em características morfológicas e bioquímicas. O criptorquidismo é uma condição patológica congênita na qual os testículos e epidídimos permanecem alojados na cavidade abdominal do organismo, causando a interrupção da espermatogênese e a ausência de espermatozóides no epidídimo, bem como atrofia e alterações histológicas que comprometem a função do epitélio germinativo do testículo e do epitélio secretor do epidídimo. A orquidopexia consiste no posicionamento dos testículos e epidídimos à topologia normal, contudo há ainda relatos de casos de infertilidade pós-orquidopexia. Alterações no epidídimo não revertidas pela orquidopexia poderiam ser co-responsabilizadas pelo baixo índice de fertilidade em indivíduos pós-orquidopexia. O objetivo deste estudo foi descrever em camundongos C57BL/6 as alterações histológicas e estereológicas causadas pelo criptorquidismo experimental e a possível reversibilidade destas alterações pela orquidopexia. Adicionalmente, foram determinadas e comparadas as concentrações séricas de testosterona e o número e o volume do núcleo das células de Leydig em camundongos controles, submetidos à condição de criptorquidismo experimental e à orquidopexia. A condição criptorquídica causou reduções significativas nos parâmetros estereológicos avaliados, contudo as alterações epididimárias foram menos acentuadas do que as verificadas para testículos. Após a orquidopexia, os valores de todos os parâmetros no epidídimo atingiram valores significativamente semelhantes aos controles. A dosagem sérica de testosterona não foi significativamente diferente em nenhum grupo analisado. A persistência da redução no número de células de Leydig na condição criptorquídica associada à não variação dos níveis de testosterona sugerem um mecanismo compensatório da atividade das células de Leydig. Os resultados demonstram que as alterações epididimárias causadas pelo criptorquidismo podem ser revertidas pela orquidopexia. Assim, a orquidopexia pode ser considerada um método eficaz para restauração da espermatogênese e alterações histológicas da cabeça do epidídimo causadas pelo criptorquidismo e, desta forma, sugere-se que a infertilidade, pós-orquidopexia, não deve ser correlacionada às alterações sofridas pela cabeça do epidídimo.

ABSTRACT

The mammalian epididymis, through secretion of androgen-dependent proteins, is responsible for maturation of the sperm aiming fecundation process, modifying the sperm in their morphological and biochemical features. Cryptorchidism is a pathological condition in which testis and epididymis are retained in the abdominal cavity, resulting in interruption of the spermatogenesis and absence of sperm in the epididymis as well as atrophy and histological alterations that compromise the function of germinative epithelium of the testicle and secretor epithelium of epididymis. Orquidopexy restored the normal topology of testis and epididymis, however there are relates of cases of infertility pos-orchidopexy. Epididymis alterations not reverted by orchidopexy could be co-responsible for decrease fertility potential pos-orchidopexy. The aim of this study was to describe in mice (C57BL/6) by histology and stereological parameters the alterations induced by experimental cryptorchidism in epididymis and testis and the possible reversibility of these alterations by orchidopexy. Additionally were determined and compared serum levels of testosterone, number and nuclear volume of Leydig cells in mice controls and mice submitted to experimental cryptorchidism and orchidopexy. The cryptorchidic conditions caused significantly reduction of stereological parameters that were analyzed, however the epididymal alterations were less decreased than testicular alterations. After orchidopexy, the values of all parameters in the epididymis were significantly similar to controls. The serum testosterone levels were not significantly different among the groups analyzed. The decreased number and nuclear volume of Leydig cells in cryptorchidic conditions not associated with variations of the serum testosterone levels suggest a compensatory mechanism of the Leydig cells activity. The results showed that alterations in caput of epididymis and interruption of spermatogenesis caused by cryptorchidism can be reverted by orchidopexy, suggesting that the infertility, pos-orchidopexy, does not have to be correlated to the alterations suffered by the caput of the epididymis.

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I. INTRODUÇÃO

No processo de espermatogênese, as células germinativas são submetidas à divisão mitótica, divisão meiótica e, adicionalmente, a processos morfogênicos de diferenciação (NISHIMUNE e OKABE, 1993), resultando na formação de células altamente diferenciadas, capazes de atingir o trato reprodutor e o gameta feminino com o objetivo de restabelecer a diploidia da espécie por meio da fecundação (SALING e LAKOSKI, 1985; YANAGIMACHI, 2001).

A espermatogênese requer uma variedade de hormônios esteróides e glicoproteínas, os quais são essenciais para o funcionamento normal do epitélio germinativo do testículo (HOLDCRAFT e BRAUN, 2004). Os hormônios esteróides e glicoproteínas atuam não só na regulação do desenvolvimento das células germinativas masculinas, mas também na proliferação e no funcionamento de células somáticas que garantem a fisiologia testicular (McLACHLAN *et al.*, 2002).

Dentre as células testiculares, é possível destacar: as células intersticiais de Leydig, que primariamente possuem a função de produção de testosterona (MENDIS-HANDAGAMA, 1997); as células mióides, que circundam o túbulo seminífero proporcionando suporte biomecânico e contratilidade (MAEKAWA *et al.*, 1996) e, por fim, as células de Sertoli, as quais estão diretamente relacionadas com a proliferação e diferenciação das células germinativas, participando no suporte estrutural e nutricional da espermatogênese, dentre outras funções importantes (GRISWOLD, 1998). Cada um destes tipos celulares é alvo de um ou mais hormônios, cuja ação é essencial para a fertilidade masculina (HOLDCRAFT e BRAUN, 2004).

A testosterona e seus metabólitos, como a di-hidrotestosterona (DHT) e o estradiol, são exemplos de hormônios sexuais que participam da regulação do desenvolvimento

gonadal a partir de gônadas indiferenciadas (imaturas), bem como do desenvolvimento das células germinativas de ambos os sexos, incluindo a diferenciação sexual masculina (McLACHLAN *et al.*, 2002; HOLDCRAFT e BRAUN, 2004).

O processo de diferenciação das células germinativas em espermatozóides envolve tanto uma fase gonadal, que ocorre no túbulo seminífero, como uma fase extra-gonadal, que ocorre no epidídimo, ducto deferente e trato reprodutor feminino (DENIS, 1994). Embora a divisão meiótica esteja completa no testículo, os espermatozóides do fluido testicular que atingem o epidídimo são pouco móveis e incapazes de fecundar *in vitro* (HAMIL *et al.*, 2000).

Dentre os ductos pelos quais os espermatozóides passam até atingir o gameta feminino, o epidídimo é o responsável por propiciar um micro-ambiente especializado no qual os espermatozóides desenvolvem elevado grau de diferenciação fenotípica e funcional (BLANCHARD e ROBAIRE, 1997). Esse órgão é altamente compartimentalizado em suas atividades (absorção e secreção) e composição celular. A variação de compartimentalização dos diferentes segmentos do epidídimo (segmento inicial, cabeça, corpo e cauda) indica que o epitélio ao longo do ducto possui diferentes funções e/ou estão sob o controle de diferentes fatores regulatórios. Segundo Blanchard e Robaire (1997), dentre esses fatores encontram-se os andrógenos que atuam em diferentes regiões e em diferentes tipos de células epiteliais do ducto epididimário.

A diferenciação do epitélio pseudo-estratificado do epidídimo inicia-se durante a segunda semana de desenvolvimento pós-natal, sendo em número de cinco os tipos celulares distintos que compõem este epitélio: células principais, basais, apicais, claras e, por fim, células halo (ROBAIRE *et al.*, 2000). Resumidamente, as células principais são as mais abundantes ao longo de todo o ducto epididimário e apresentam longos estereocílios relacionados com a secreção e absorção de proteínas (CUMMINS *et al.*, 1986;

FOUCHECOURT *et al.*, 2003). As pequenas células basais, mais numerosas no segmento inicial e no corpo, parecem atuar como possíveis precursoras das demais células epiteliais, bem como na produção de anti-oxidantes (CALVO *et al.*, 1997). As células apicais são homólogas às principais e parecem estar envolvidas no transporte e digestão de proteínas e carboidratos do lúmen (SYNTIN *et al.*, 1999). As células halo correspondem a linfócitos intra-epiteliais e as células claras, igualmente distribuídas ao longo de todo o epitélio epididimário, apresentam funções ainda controversas, envolvendo o processo de endocitose ou a acidificação do fluido epididimário (FLICKINGER *et al.*, 1978; YEÜNG *et al.*, 1994; FLESCHE e GADELLA, 2000; ROBAIRE *et al.*, 2000; FRANÇA *et al.*, 2005).

Durante a passagem do espermatozóide pelo epidídimo, a composição protéica de sua superfície pode ser alterada ou extraída, devido à atividade secretora de proteínas e enzimas pelo epitélio epididimário (FLESCHE e GADELLA, 2000; ARROTÉIA *et al.*, 2004; DACHEUX *et al.*, 2005). Estas alterações bioquímicas, conjuntamente identificadas como maturação epididimária, permite ao gameta potencializar sua motilidade, estabilizar a cromatina e adquirir sítios para reconhecimento e ligação de moléculas da zona pelúcida, bem como para a fusão com a membrana plasmática do oócito (MOORE, 1998; KAUNISTO *et al.*, 1999; FLESCHE e GADELLA, 2000; DACHEUX, 2002).

No que diz respeito ao papel secretor cerca de 75% das proteínas que participam da maturação epididimária são secretadas nas regiões iniciais deste ducto (segmento inicial e cabeça do epidídimo), sendo que 48% das proteínas secretadas pela cabeça do epidídimo são dependentes de andrógenos (SYNTIN *et al.*, 1999, ROBAIRE *et al.*, 2000; TOSHIMORI, 2003; DACHEUX *et al.*, 2005). Muitas destas proteínas correspondem às enzimas capazes de alterar glicoproteínas de superfície do espermatozóide e, após liberadas na luz do epidídimo, parecem ligar-se à superfície do espermatozóide (VREEBURG *et al.*, 1992;

TULSIANI *et al.*, 1993; EDDY e O'BRIEN, 1994; KIRCHHOFF e HALE, 1996) ou mesmo no interior da vesícula acrossômica (COHEN *et al.*, 2000).

A atividade secretora (porcentagem de secreção) de natureza protéica no epidídimo decresce ao longo do ducto, de modo que pouquíssimas proteínas (cerca de 1% do total de proteínas) são secretadas especificamente na região da cauda (DACHEUX *et al.*, 2005). Segundo Robaire *et al* (2000), este fenômeno se deve ao fato de que as proteínas secretadas nos segmentos mais proximais do epidídimo são acumuladas no fluido luminal ao longo da extensão do ducto, promovendo a maturação gradativa dos espermatozóides; desta maneira, a cauda do epidídimo apresenta como função principal, a estocagem e proteção dos espermatozóides (GATTI *et al.*, 2004).

O criptorquidismo é uma doença congênita na qual os testículos permanecem alojados na cavidade abdominal do organismo. Desta forma, ficam submetidos às temperaturas de 2 a 6 °C, superiores à temperatura do escroto (FORESTA *et al.*, 1996; GUNAY *et al.*, 1998; HUTSON e HASTHORPE, 2005). A perda da regulação térmica causada pelo criptorquidismo resulta em interrupção da espermatogênese (BRONSON e HEIDMAN 1993; FORESTA *et al.*, 1996; BASIMOGLU-KOCA *et al.*, 1998; ARROTÉIA *et al.*, 2005; HUTSON e HASTHORPE, 2005). Este fenômeno impossibilita o desenvolvimento das células germinativas testiculares e a chegada de espermatozóides no epidídimo. Cerca de 33% dos recém-nascidos prematuros e 3% dos recém-nascidos a termo possuem testículos criptorquídeos (IVELL e HARTUNG, 2003). Após 1 ano de idade, essa porcentagem cai para 1% espontaneamente, prevalecendo a mesma para a população adulta (KUNEJ *et al.*, 2003). De modo geral, nos indivíduos adultos, a persistência do criptorquidismo está associado à infertilidade, principalmente se este for bilateral (NISHIMUNE *et al.*, 1986; BRONSON e HEIDMAN 1993; KOCAK *et al.*, 2001; BRAZ, 2002; COROPPO *et al.*, 2005; HUSMANN,

2005). O índice de infertilidade é de 32% em homens com criptorquidismo unilateral e cerca de 59% em homens com criptorquidismo bilateral; em casais inférteis, o histórico clínico revela que 8% dos homens apresentaram testículos criptorquídicos (NIESCHLAG *et al.*, 2000).

A causa do criptorquidismo é multifatorial e em alguns aspectos desconhecida, contudo as anormalidades endócrinas e anatômicas, os fatores genéticos e o micro ambiente em que ocorre a diferenciação do testículo podem ser responsáveis por esta patologia (KUNEJ *et al.*, 2003; HUTSON e HASTHORPE, 2005).

Do ponto de vista estrutural, testículos criptorquídicos apresentam-se atróficos (MININBERG e SCHLOSSBERG, 1983; MILIARAS *et al.*, 1997; ZHANG, 2002; ARROTÉIA *et al.*, 2005). Histologicamente, em situações de criptorquidismo, as alterações dos tecidos constituintes do epidídimo e vasos deferentes são pobremente descritas. No epidídimo, foram descritos o estado indiferenciado do epitélio, a ausência de espermatozóides no lúmen, a desorganização estrutural do ducto e a ausência da expressão de proteínas de superfície, localizadas no ápice, do epitélio e do espermatozóide (JOHANSEN, 1987; DePALMA *et al.*, 1988; MILIARAS *et al.*, 1997; De MIGUEL *et al.*, 2001; ARROTÉIA *et al.*, 2005).

Tratamentos hormonais e cirúrgicos, este último denominado orquidopexia, foram desenvolvidos para o posicionamento do testículo e ductos associados à topologia normal (ZITZMANN e NIESCHLAG, 2000; NGUYEN *et al.*, 2002; ZHANG, 2002). Dois terços dos casos de testículos localizados no canal inguinal respondem positivamente à estimulação do eixo hipotalâmico-pituitário-gonadal com os hormônios hCG (Human chorionic gonadotropin) e/ou o hormônio GnRH (Gonadotrophin-releasing hormone), contudo mais de 85% de casos

com testículos na cavidade abdominal não apresentam resposta positiva à esta estimulação (IVELL e HARTUNG, 2003).

A orquidopexia tem sido relatada como uma técnica eficaz na restauração da espermatogênese em procedimento clínicos e experimentais (ZITZMANN e NIESCHLAG, 2000; NGUYEN *et al.*, 2002) e consiste no posicionamento dos testículos para o escroto, através do canal inguinal. A orquidopexia, quando realizada na infância, permite a recuperação do número normal de espermatozóides em 20% dos casos de criptorquidismo bilateral e em 70% dos casos de criptorquidismo unilateral, considerando que apenas 80% dos homens em geral possuem um número normal de espermatozóides no ejaculado (JÉGOU *et al.*, 1984; CORTES, 1998).

A orquidopexia tem como objetivo, entre outros, minimizar as chances de desenvolvimento de câncer testicular (BERKMEN e ALAGOL, 1998; HUSMANN, 2005), reduzir os riscos de infertilidade, evitar possíveis hérnias, reposicionar os testículos em uma região mais facilmente palpável (facilitando exames clínicos) e melhorar a estética e as questões psicológicas decorrentes ao quadro de criptorquidismo (ZITZMANN e NIESCHLAG, 2000). O procedimento cirúrgico culmina em melhores resultados quando realizado em pacientes entre 6 a 24 meses de vida. PILLAI e BESNER (1998) relataram que 95% dos pacientes submetidos à orquidopexia por criptorquídia unilateral foram diagnosticados como férteis em comparação a somente 30%-50% dos pacientes não tratados com este procedimento.

Existe um consenso quanto aos danos causados pelo criptorquidismo à espermatogênese e quanto à reversibilidade proporcionada pela orquidopexia, mas a extensão do dano ou a proporcionalidade desta recuperação não estão plenamente esclarecidas (ZHANG *et al.*, 2002). Casos de infertilidade masculina pós-orquidopexia são

relatados na literatura (De PALMA *et al.*, 1988; PALMER, 1991; ZHANG, 2002), sugerindo que anormalidades estruturais nos ductos espermáticos poderiam coexistir com uma espermatogênese normal pós-orquidopexia (MOLLAEIAN *et al.*, 1994; NEGRI *et al.*, 2003).

Resultados de nosso laboratório demonstraram que camundongos submetidos à condição de criptorquidismo experimental durante um período 90 dias apresentaram significativa atrofia dos túbulos seminíferos e alterações no epitélio epididimário. No entanto, 30 dias pós orquidopexia parecem ser suficientes para a recuperação da maioria dos túbulos seminíferos e da maior parte do epitélio epididimário anteriormente atroficos (ARROTÉIA, *et al.*, 2005). Contudo, nenhuma análise estereológica foi realizada para se avaliar a significância destes resultados e correlacionar a dosagem sérica de testosterona com a estrutura testicular (células de Leydig) e com a região da cabeça do epidídimo.

Assim, o objetivo deste estudo foi através da estereologia e dosagem sérica de testosterona, verificar as alterações estruturais no epidídimo causadas pelo criptorquidismo e a possível reversibilidade destas alterações pela orquidopexia, bem como correlacionar estes achados com os níveis de testosterona.

Nesta investigação priorizou-se o estudo da cabeça do epidídimo, uma vez que esta região apresenta a maior atividade de secreção de proteínas importantes para a maturação epididimária do espermatozóide (SYNTIN *et al.*, 1999, ROBAIRE *et al.*, 2000; DACHEUX *et al.*, 2005). Animais imaturos foram utilizados na tentativa de mimetizar ao máximo a situação do criptorquidismo congênito, uma vez que o primeiro contato do espermatozóide com o lúmen do epidídimo ocorre durante a segunda semana do desenvolvimento pós natal.

II. OBJETIVOS

Os objetivos deste trabalho foram:

1. Descrever as alterações histológicas (análise qualitativa) e avaliar por métodos estereológicos (análise quantitativa) as alterações epididimárias causadas pelo criptorquidismo experimental e a possível reversibilidade destas alterações pela orquidopexia;
2. Determinar e comparar as concentrações séricas de testosterona em camundongos controles, camundongos submetidos à condição de criptorquidismo experimental e camundongos submetidos à orquidopexia. Correlacionar a concentração sérica de testosterona com o binômio alteração/regeneração da cabeça do epidídimo e número e o volume nuclear das células de Leydig.

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III- ARTIGO

HISTOLOGICAL AND STEREOLOGICAL ANALYSIS OF EPIDIDYMIS AND LEYDIG CELLS IN MICE SUBMITTED TO EXPERIMENTAL CRYPTORCHIDISM AND ORCHIDOPEXY

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ABSTRACT

Cryptorchidism is a pathological condition in which the testes are retained in the abdominal cavity, resulting in atrophy and the interruption of spermatogenesis. Histological alterations have been described in the epididymis of cryptorchidic testes. Orchidopexy restores testicular spermatogenesis in experimental and clinical procedures, but it is still unclear whether the histological changes in the epididymis caused by cryptorchidism can be reverted by orchidopexy. The aim of this study was to use stereological analysis to evaluate the testicular and epididymal changes in immature mice following cryptorchidism and to determine whether the alterations could be reversed by orchidopexy. In addition, the number and nuclear volume of Leydig cells and the serum testosterone levels were determined. There was a significant reduction in all testicular and epididymal parameters analyzed in cryptorchidic mice, with a significant recovery in mice submitted to orchidopexy. The reduction in the number and nuclear volume of Leydig cells in cryptorchidic and orchidopexic mice associated with normal serum testosterone levels suggested a compensatory mechanism in the activities of Leydig cells. These findings indicate that orchidopexy restores the histological and stereological alterations caused by cryptorchidism and suggest that post-orchidopexic infertility does not result from the testicular and epididymal alterations caused by cryptorchidism.

KEY WORDS

Cryptorchidism, Epididymis, Leydig cells, Orchidopexy, Testis

INTRODUCTION

Cryptorchidism is the failure of the testes to descend into the scrotum. In this pathological condition, the testes are retained in the abdominal cavity and the seminiferous tubules become atrophic as a result of the increase in temperature, which is unfavorable for spermatogenesis (IVELL and HARTUG, 2003). The incidence of cryptorchidism is ~1-4% in male neonates (TOPPARI *et al.*, 2001). The cause of cryptorchidism is multifactorial, although

possible causes and risk factors such as endocrine disorders, anatomical abnormalities and environmental and genetic factors can explain the aetiology of this phenomenon (NIESCHLAG *et al.*, 2000). Some gross abnormalities have been described in the epididymal tissue of humans with cryptorchidic testis but, in contrast to the testes, the histological status of the epididymis in this population of patients has not received similar attention. Histological analysis of the epididymis in cryptorchidic mammals has confirmed the immaturity of these organs, with the possibility that these alterations may adversely affect the potential for fertility (BASIMOGLU-KOCA *et al.*, 1998; De MIGUEL *et al.*, 2001, ARROTÉIA *et al.*, 2005). In addition, two important complications of cryptorchidism are an increased risk of developing testicular cancer and a decreased sperm count and infertility (KUNEJ *et al.*, 2003). The role of the epididymis in humans is still unclear, although in all other species this organ is essential for sperm maturation and storage once these have left the germinal epithelium (IVELL and HARTUG, 2003; DACHEUX *et al.*, 2005).

Cryptorchidism can be treated with hormonal therapy or surgery. However, since the response to hormonal therapy is variable and is not durable, surgical treatment is frequently recommended (MEIJER *et al.*, 2001). Orchidopexy is one of the most commonly performed operations in pediatric surgery, with the undescended testes, epididymis and vas deferens being replaced in the scrotum (HEALTH *et al.*, 1984; ZHANG *et al.*, 2002; ARIDOGAN *et al.*, 2003). The typical age for orchidopexy has gradually decreased in an attempt to reduce the index of infertility, but many patients more than 4 years old still undergo the procedure (LAMAH *et al.*, 2001; TOLEDANO *et al.*, 2003).

Despite general agreement that cryptorchidism leads to spermatogenic damage and causes epididymal alterations, and that orchidopexy improves spermatogenesis, it is still unclear whether the histological changes in the epididymis caused by cryptorchidism can be reverted (ZHANG *et al.*, 2002).

In addition, despite the essential role of testosterone in maintaining normal spermatogenesis, there is currently little quantitative data on serum testosterone concentrations and their relationship to the number of Leydig cells in unilateral and bilateral cryptorchidism and orchidopexy (JEGOU *et al.*, 1984; WEINBAUER and NIESCHLAG, 1997).

The aim of this study was to histologically and stereologically evaluate the changes in the caput segment of the epididymis following uni- and bilateral cryptorchidism in mice

(C57BL6) and to investigate their reversibility by orchidopexy. In addition, the number and nuclear volume of Leydig cells and the serum testosterone levels were determined to examine their correlation with the stereological changes in the epididymal caput.

MATERIALS AND METHODS

ANIMALS

Seventy 15-day-old-mice (C57BL6) were housed at $23 \pm 2^{\circ}\text{C}$ on a light/dark cycle of 12 h with lights on at 6 a.m. and free access to pelleted laboratory chow and water. The mice were randomly allocated to one of five groups:

Group I: intact 15-day-old-mice (n=10) (immature control mice)

Group II: intact 105-day-old-mice (n=10) (mature control mice)

Group III: intact 195-day-old-mice (n=10) (mature control mice)

Group IV: 15-day-old mice subjected to unilateral (n=10) and bilateral (n=10) surgical cryptorchidism and sacrificed after 90 days at the age of 105 days (cryptorchidic mice).

Group V: 15-day-old mice subjected to unilateral (n=10) and bilateral (n=10) surgical cryptorchidism followed by orchidopexy at 105 days of age and sacrificed at 195 days of age (orchidopexic mice).

This study was approved by the institutional Committee for Ethics in Animal Experimentation (protocol number 855-3). The NIH guidelines for the care and use of laboratory animals were followed.

SURGICAL PROCEDURE FOR EXPERIMENTAL CRYPTORCHIDISM

For all surgical procedures, the mice were anaesthetized with ketamine (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Surgery was done under aseptic conditions according to a procedure described by (NISHIMUNE *et al.*, 1978). Experimental cryptorchidism was produced following a lateral laparotomy. The adipose tissue around the epididymis was pulled from the scrotum with tweezers and then sutured to the abdominal muscle wall. For unilateral cryptorchidism, only the right testis and epididymis were manipulated. The testes, epididymis and blood samples were collected from mice three months after producing experimental cryptorchidism.

SURGICAL PROCEDURE FOR EXPERIMENTAL ORCHIDOPEXY

Three months after producing cryptorchidism, the mice were anaesthetized and a lateral laparotomy was done to allow the adipose tissue of the testis to be released from the abdominal wall and pushed back into the scrotum (NISHIMUNE *et al.*, 1978). The testis, epididymis and blood samples were collected three months after orchidopexy.

TISSUE PROCESSING

Five mice of each group were anaesthetized and perfused via the left ventricle with 4% paraformaldehyde in 0.2 M phosphate buffered saline (PBS, pH 7.4). The testes and epididymis were immediately removed and fixed in the same solution for 4 h and processed followed by embedding in paraffin and sectioning (5 µm thick). Deparaffinized sections were stained with haematoxylin-eosin and examined by light microscopy.

STEREOLOGICAL ANALYSES

The testes and epididymis were weighed (SA 210 precision balance with a precision of 0.1 mg), and the total testicular and epididymal volumes were determined as described elsewhere (SHERLE, 1970).

All stereological analyses were done using an image analyzer (Image Pro-Plus, version 4.1.12) and a representative transverse section of the epididymal caput and testis from each mouse. For each mouse, 30 cross-sectioned tubules were randomly selected and measured using 20X and 60X objective lenses for the epididymis and testis. In each epididymal duct, the parameters measured included total duct diameter, height of epithelium, total duct area, luminal area, and epithelial area, which was calculated as the difference between the total duct area and the luminal area of each epididymal duct. Leydig cells were identified in transverse sections of the testis and their diameter and nuclear area were measured with a 60X objective lens.

The volume of the tissue components (total tubule, epithelial, luminal and interstitial volumes) in the testis and epididymal caput was estimated as a percentage using a 100 point reticle on images obtained with a 20X objective lens. The volume of the tissue components

(expressed as a percentage) and the total volume of the testis and epididymis were used to determine the absolute values of the tissue components of the testis and epididymis.

The nuclear volume of a single Leydig cell was estimated using the formula for a sphere: $\text{volume} = 4/3\pi r^3$, where r corresponds to the radius (= diameter/2) of the nucleus. The total nuclear volume of Leydig cells in the testis was calculated using a 100 point reticle on images obtained with a 20X objective lens in 30 round cross-sections of tubules. The number of Leydig cells was then calculated as (CASTRO *et al.*, 2002):

$$\text{Number of Leydig cells} = \frac{\text{Total nuclear volume of Leydig cells in the testis}}{\text{Nuclear volume of a single Leydig cell}}$$

The microscope was calibrated using a graduated slide prior to obtaining the epididymal and testicular measurement at the same magnification. The results were expressed in μm^2 for areas, in μm for height and diameters, and in μl for volume.

QUANTIFICATION OF TESTOSTERONE

Five mice in each group were anaesthetized and sacrificed by decapitation, and blood was collected and centrifuged at 1,600 g for 20 min at 4°C. The serum was separated and stored at -70°C until required. The serum levels of testosterone were measured using an electrochemiluminescence immunoassay (ECL) and evaluated analytically in a Modular E170 immunoanalyzer (Roche Diagnostics), as previously described (REYNDERS *et al.*, 2005).

STATISTICAL ANALYSIS

The results were expressed as the mean $\pm$ S.E.M. All measurements were compared statistically using one-way analysis of variance (ANOVA) followed by Tukey's mean comparison test. The level of significance was set at 5% ($p < 0.05$). The statistical analyses were done using the statistical program INSTAT v 2.01 (GraphPad, San Diego, CA, USA).

RESULTS

HISTOLOGICAL ANALYSIS

The testes in Group I was devoid of sperm and the seminiferous epithelium consisted of some spermatogonies, spermatocytes and Sertoli cells (Figure 1a). The Leydig cells in this group were morphologically indistinguishable from fibroblast-like cells or mesenchymal cells in the testicular interstitium (PARK and JAMESON, 2005) (Figure 1a inset). The epithelial cells in the epididymal caput showed undifferentiated characteristics, including a small height and juxtaposition of the cells (Figure 1b).

In Groups II and III, the testes contained seminiferous tubules with complete spermatogenesis and the germ cells were in various stages of development, with spermatozoa in the tubule lumina (Figure 1c). There were numerous Leydig cells in the intertubular compartment (Figure 1c, inset). In the caput segment of the epididymis, principal, apical and basal cells were seen in the columnar pseudostratified epididymal epithelium. The lumen contained numerous spermatozoa (Figure 1d).

In Group IV (unilateral and bilateral cryptorchidic mice), the cryptorchidic testes had an atrophic gonad and the seminiferous tubules were completely devoid of spermatocytes, spermatids and spermatozoa; these cells were replaced by several large stores. The seminiferous epithelium was lined by spermatogonia, Sertoli cells and cells in degeneration (Figure 1e), and Leydig cells were abundant in the intertubular tissue (Figure 1e, inset). The epididymis of cryptorchidic mice was elongated and flattened when compared with that of Group II and III mice. The histological structures of the epididymis were altered to varying degrees: the epithelial cells were flattened and narrowed in the focal areas, there was abundant interstitial tissue and disorganization of the tubular arrangement, and spermatozoa were generally absent (Figure 1f).

Full spermatogenesis was restored in the testes of Group V mice that were initially unilaterally or bilaterally cryptorchidic and then made orchidopexic (Figure 1g); there was no obvious structural difference when these mice were compared to the animals in Groups II and III (Figure 1c). The Leydig cells were distributed in the intertubular tissue (Figure 1g, inset). The epididymal caput of cryptorchidic testes rendered orchidopexic showed a recovery in the tubular arrangement, with the lumen containing plenty of sperm, and the differentiated

columnar pseudostratified epithelium contained principal, basal and apical cells (Figure 1h) similar to those of Groups II and III (Figure 1d).

In unilaterally cryptorchidic mice, the contralateral intact epididymis had histological features similar to those of Groups II and III, whereas the epididymis of the cryptorchidic testis showed the same alterations seen in epididymis from bilaterally cryptorchidic mice.

STEREOLOGICAL ANALYSIS

Cryptorchidic testicular weight decreased significantly to 22.5% and 28.5% of the weight in Groups II and III, respectively. Epididymal weight from cryptorchidic mice decreased significantly to 61.3% and 55.3% of the weight in Groups II and III, respectively. There was no significant difference in the testicular and epididymal weight after orchidopexy compared to Groups II and III (Table I).

Stereological measurements indicated a significant reduction ($p < 0.05$) in the total testicular volume (Fig. 2a), seminiferous tubule volume, seminiferous epithelium volume and lumen volume in cryptorchidic mice (Table I). The volumes were equivalent to those of Group I, except for that of interstitial tissue, which was not significantly different. However, in mice subjected to orchidopexy, the testes showed full development with normal spermatogenesis and no significant stereological alterations in the parameters analyzed (Table I), although the total testicular volume was about 83% of that of Groups II and III; however, this difference was not significant (Fig. 2a).

The cryptorchidic epididymis also showed atrophy, with the total volume (Fig. 2b), the volume of the epididymal epithelium and the volume of the epididymal lumen being significantly reduced (Table I). Compared to Groups II and III, the epididymal interstitial volume was significantly reduced only in unilaterally cryptorchidic mice. In addition, the total duct diameter was significantly smaller than in the control groups. There was no significant difference in the height of the epididymal epithelium in cryptorchidic mice (Table I). There was a significant reduction ($p < 0.05$) in the total tubular area, luminal area and epithelial area in the epididymal duct of cryptorchidic mice (Fig. 2c-e).

After orchidopexy, all of the parameters measured were equal or smaller than in Groups II and III, but these differences were not significant (Figure 2, Table I), indicating that the epididymal alterations caused by cryptorchidism had been reverted.

In unilaterally cryptorchidic mice, the contralateral intact epididymis had morphometric parameters similar to those of Groups II and III, whereas the epididymis of cryptorchidic testes showed the same changes as epididymis from bilaterally cryptorchidic mice.

LEYDIG CELLS AND TESTOSTERONE LEVELS

The number of Leydig cells in cryptorchidic mice decreased significantly to 14.8% of the values in Groups II and III whereas after orchidopexy, the number of Leydig cells was 55.7% of those of Groups II and III (Table I). The nuclear volume of Leydig cells in cryptorchidic mice decreased significantly to 15.3% of the values in Groups II and III, and orchidopexy restored this volume to 65.3% of the values in these two groups (Figure 2f).

Although testosterone levels were reduced in uni and bilateral cryptorchidism, the differences among the four groups (II, III, IV and V) were not significant (Figure 2g).

DISCUSSION

In this study, we used stereological analyses of area, volume, height and diameter to assess the structural changes in the epididymis following experimental uni- and bilateral cryptorchidism and to determine whether these alterations could be reversed by orchidopexy. In addition, Leydig cells were analyzed stereologically (volume and number of cells) and the testosterone levels were measured. The experimental cryptorchidic group consisted of 15-day-old because spermatogenesis in these mice is blocked before completion of the first wave of germ cell differentiation, as occurs in the epididymis of newborn cryptorchidic boys (ARROTÉIA *et al.*, 2005).

Cryptorchidism produced testicular and epididymal damage in immature mice, including atrophy of the testes and epididymis. The seminiferous tubules contained many empty spaces, indicating severe disruption of germ cell development. The epididymal epithelium became cubical and undifferentiated, and there were no sperm in the lumen, as previously described (JEGOU *et al.*, 1984; BASIMOGLU-KOCA, *et al.*, 1998; BILINSKA *et al.*, 2003; BARTHOLD and GONZALEZ, 2003; IVELL and HARTUG, 2003; ARROTÉIA *et al.*, 2005).

The stereological analyses showed a significant reduction in all of the parameters analyzed in the cryptorchidic groups when compared with the control groups. In general, the

values of the parameters analyzed in the testes decreased to 15%-25% of those in Groups II and III, while in the epididymis the values were 40%-87% of those in Groups II and III, suggesting that the testes were more temperature-sensitive than the epididymis.

According to DeMIGUEL *et al.* (2001), the total area and total diameter of the duct in the caput segment of the epididymis in childhood and adulthood were smaller in cryptorchidism than in the corresponding controls. However, the caput luminal area was not significantly different in individuals with cryptorchidism versus normal individuals at any age. As seen here in Group IV there was a significant reduction in the total area and total diameter of the duct, and the caput luminal area was significantly smaller than in Groups II and III. The variability seen in some of the parameters in our study may be a consequence of the experimental model used for cryptorchidism and age of the mice when cryptorchidism was induced. In agreement with this conclusion, NISHIMUNE *et al.* (1978) reported that mature rats responded differently to cryptorchidism compared to immature rats and mature mice. In addition, BARTHOLD and REDMAN (1996) and ARRÓTEIA *et al.* (2005) observed that epididymal abnormalities were more evident in immature cryptorchidic mice than in mature cryptorchidic mice, probably because of a delayed epididymal development in the former animals.

Although the developmental alterations in the human cryptorchidic epididymis have not been studied in depth, in cryptorchidic children 7–10 years old the epididymis can show marked underdevelopment (ZONDEK and ZONDEK, 1980) and irreversible structural alterations (DePALMA *et al.*, 1988; BARTHOLD and REDMAN, 1996; MILIARAS *et al.*, 1997; BASIMOGLU-KOCA *et al.*, 1998; DeMIGUEL *et al.*, 2001). Since the epididymis is important in sperm maturation (KIRCHHOFF *et al.*, 1998; MOORE, 1998; ROBAIRE *et al.*, 2000), the possibility of reversing the histological alterations caused by cryptorchidism is of clinical relevance.

According to DePALMA *et al.* (1988), it is unclear whether the histological changes seen in human epididymis would disappear with orchidopexy. Despite the suggestion of DeMIGUEL *et al.* (2001) that surgical descent of the testes would not completely reverse the epididymal histological alterations associated with cryptorchidism, we have shown here that the epididymis of experimentally cryptorchidic immature mice developed normally after surgical intervention. Indeed, after orchidopexy, all of the testicular and epididymal

parameters analyzed showed a significant recovery. In general, the parameters analyzed in orchidopexic testes recovered to within 55%-83% of the control values while in the epididymis the values recovered to 79%-100% of the control values; these findings suggested that the testes are more temperature-sensitive than the epididymis. Thus, replacement of the testis and epididymis in the scrotum reestablishes an appropriate environment for spermatogenesis and differentiation of the epididymal epithelium.

The number and nuclear volume of Leydig cells per testis was significantly reduced in the cryptorchidic and orchidopexic groups, particularly in the former. The greater effect of cryptorchidism may reflect the fact that this intervention was done when the mice were 15-days-old and may have influenced the differentiation of the Leydig cells. Although testosterone levels were reduced in bilateral cryptorchidism, none of the changes were significant among any of the groups, thus indicating that Leydig cell function was unchanged in these experimental conditions (JEGOU *et al.*, 1984). The reduction in the number of Leydig cells in cryptorchidic mice and the normal serum testosterone level suggests a compensatory mechanism for maintaining Leydig cell activities.

In conclusion, cryptorchidism can result in less than ideal conditions that alter the histological, stereological structure and hormonal parameters of the testes and epididymis. However, orchidopexy was able to maintain the secretory activities of the epididymal caput (ARROTÉIA *et al.*, 2004) and restore spermatogenesis and normal features of the epididymis.

LEGENDS, FIGURES AND TABLE.

Figure 1- Photomicrographs of testes (a, c, e and g), epididymal caput (b, d, f and h) and Leydig cells (inset - a, c, e and g). Group I (a-b), Group III (c-d), Group IV (e-f) and Group V (g-h). Scale bar: a–h: 30 μ m (20x objective lens); inset: 60x objective lens. Testes from Group I were (a) devoid of sperm, inset (a) shows that Leydig cells in Group I were morphologically indistinguishable from fibroblast-like cells or mesenchymal cells in the testicular interstitium and (b) the epididymal epithelium was undifferentiated. Testes from Group III (c) showing sperm in the lumen; inset (c) shows differentiated Leydig cells and (d) the epididymal epithelium was completely differentiated. Testes from Group IV (e) showing atrophic seminiferous tubules with interruption of spermatogenesis; inset (e) shows differentiated Leydig cells; the arrangement of the tubular sections of the epididymis (f) was irregular, with abundant interstitial tissue and incompletely differentiated epithelial cells. Group V (g) shows restored normal spermatogenesis; inset (g) shows differentiated Leydig cells. The normal morphological/histological appearance of the epididymis was restored (h).

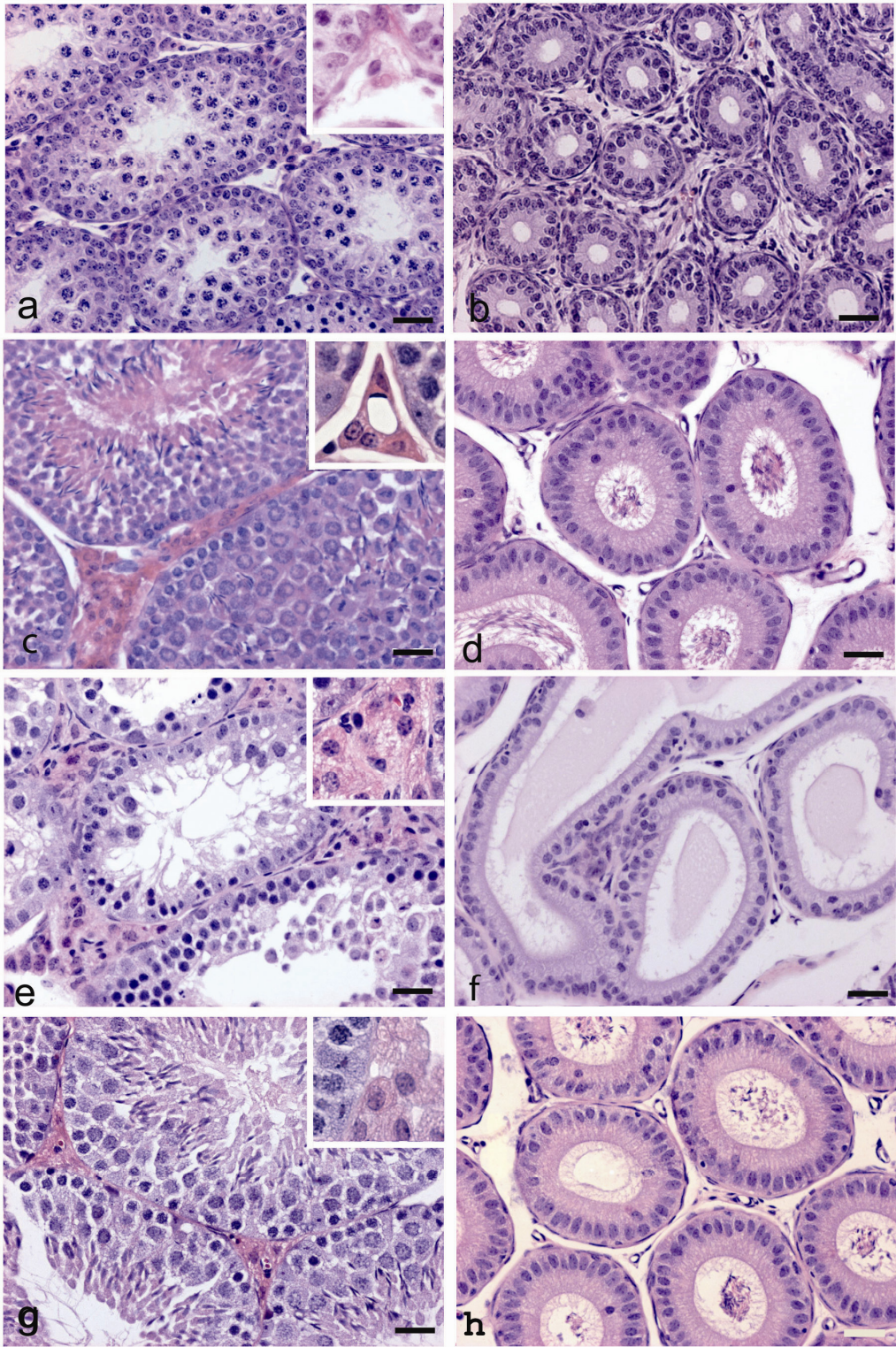


Table I- Quantitative data (mean $\pm$ SEM) for testes and epididymis. Groups I, II and III are control mice, Group IV (unilateral, contralateral and bilateral) corresponds to cryptorchidic mice, and Group V (unilateral, contralateral and bilateral) represents orchidopexic mice.

Parameters	Group I	Group II	Group IV			Group III	Group V		
			Unilateral	Contralateral	Bilateral		Unilateral	Contralateral	Bilateral
Weight (mg)									
Testicle	12 $\pm$ 0.54 ^a	120.9 $\pm$ 2.20 ^b	27.3 $\pm$ 1.42 ^c	118.5 $\pm$ 0.40 ^b	33.6 $\pm$ 1.70 ^c	122.0 $\pm$ 6.05 ^b	100.8 $\pm$ 6.67 ^d	115.9 $\pm$ 1.46 ^{bd}	116.7 $\pm$ 4.74 ^{bd}
Epididymis	3.2 $\pm$ 0.55 ^a	44.4 $\pm$ 1.86 ^b	24.5 $\pm$ 3.36 ^c	45.3 $\pm$ 3.02 ^b	29.9 $\pm$ 0.52 ^c	49.2 $\pm$ 3.29 ^b	46.5 $\pm$ 1.94 ^b	52.5 $\pm$ 2.10 ^b	52.1 $\pm$ 2.29 ^b
Vol. per organ (μl)									
Testicle									
Sem. Tubules	9.6 $\pm$ 0.50 ^a	96.81 $\pm$ 2.84 ^b	13.3 $\pm$ 1.38 ^a	85.46 $\pm$ 0.95 ^b	19.9 $\pm$ 1.84 ^a	93.7 $\pm$ 6.50 ^b	79.73 $\pm$ 5.14 ^b	86.3 $\pm$ 1.98 ^b	87.8 $\pm$ 3.32 ^b
Sem. Epithel.	7.5 $\pm$ 0.69 ^a	77.07 $\pm$ 3.34 ^b	9.41 $\pm$ 0.89 ^a	57.40 $\pm$ 1.12 ^c	14.6 $\pm$ 1.39 ^a	69.1 $\pm$ 4.90 ^{bc}	54.30 $\pm$ 3.89 ^c	58.7 $\pm$ 0.76 ^c	58.1 $\pm$ 2.75 ^c
Lumen	2.08 $\pm$ 0.21 ^a	20.74 $\pm$ 0.96 ^b	4.02 $\pm$ 0.67 ^a	28.06 $\pm$ 0.70 ^c	5.3 $\pm$ 1.01 ^a	24.5 $\pm$ 1.61 ^{bc}	25.43 $\pm$ 1.38 ^{bc}	27.6 $\pm$ 1.32 ^c	29.7 $\pm$ 0.74 ^c
Interstitial tissue	2.4 $\pm$ 0.23 ^a	21.89 $\pm$ 3.04 ^{bc}	13.7 $\pm$ 1.08 ^b	32.07 $\pm$ 1.29 ^d	13.4 $\pm$ 2.73 ^b	27.1 $\pm$ 2.35 ^{dc}	20.39 $\pm$ 1.67 ^{bc}	28.7 $\pm$ 0.67 ^{dc}	28.1 $\pm$ 3.08 ^{dc}
Vol. per organ (μl)									
Epididymis									
Duct Epidi	1.47 $\pm$ 0.31 ^a	30 $\pm$ 1.16 ^b	15.2 $\pm$ 0.91 ^c	32.30 $\pm$ 2.00 ^b	19.4 $\pm$ 0.31 ^c	34 $\pm$ 2.30 ^b	34.9 $\pm$ 1.48 ^b	37.0 $\pm$ 2.06 ^b	36.3 $\pm$ 2.44 ^b
Duct Epithel	1.34 $\pm$ 0.29 ^a	23 $\pm$ 0.85 ^b	13.3 $\pm$ 0.75 ^c	25.81 $\pm$ 1.38 ^b	15.9 $\pm$ 0.33 ^c	22 $\pm$ 1.74 ^b	27.0 $\pm$ 1.26 ^b	29.2 $\pm$ 1.44 ^b	29.2 $\pm$ 1.68 ^b
Lumen	0.11 $\pm$ 0.02 ^a	6.8 $\pm$ 0.56 ^b	2.5 $\pm$ 0.38 ^{ac}	6.49 $\pm$ 0.61 ^b	3.4 $\pm$ 0.53 ^c	7.8 $\pm$ 0.73 ^b	7.9 $\pm$ 0.37 ^b	7.8 $\pm$ 0.63 ^b	7.2 $\pm$ 0.82 ^b
Interstitial tissue	1.67 $\pm$ 0.23 ^a	14.4 $\pm$ 1.22 ^b	8.4 $\pm$ 0.86 ^c	13.02 $\pm$ 2.04 ^{bc}	10.6 $\pm$ 0.32 ^{bc}	14.6 $\pm$ 0.87 ^b	11.6 $\pm$ 0.50 ^{bc}	15.5 $\pm$ 0.61 ^b	15.8 $\pm$ 1.58 ^b
Epididymis									
Diameter (μm)	39.0 $\pm$ 1.37 ^a	107.8 $\pm$ 2.58 ^b	92.5 $\pm$ 3.36 ^c	103.9 $\pm$ 2.76 ^b	96.6 $\pm$ 1.28 ^c	108.9 $\pm$ 2.82 ^b	110.6 $\pm$ 0.78 ^b	111.3 $\pm$ 1.42 ^b	115.5 $\pm$ 2.56 ^b
Epithel. height (μm)	15.4 $\pm$ 0.94 ^a	30.5 $\pm$ 0.57 ^b	27.4 $\pm$ 0.28 ^b	27.6 $\pm$ 1.15 ^b	28.3 $\pm$ 0.44 ^b	29.57 $\pm$ 0.97 ^b	29.90 $\pm$ 0.68 ^b	30.1 $\pm$ 0.86 ^b	31.55 $\pm$ 0.78 ^b
Number of Leydig cells (10⁶)	*	6.51 $\pm$ 1.10 ^a	0.85 $\pm$ 0.1 ^b	6.3 $\pm$ 0.9 ^a	0.95 $\pm$ 0.11 ^b	6.48 $\pm$ 0.7 ^a	3.53 $\pm$ 0.44 ^c	6.0 $\pm$ 0.61 ^a	3.63 $\pm$ 0.62 ^c

Epithel.: Epithelium.

Sem.: Seminiferous.

^a: Significantly equal to immature mice, p<0.05.

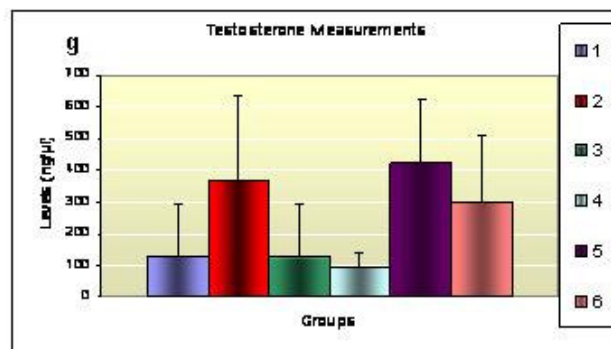
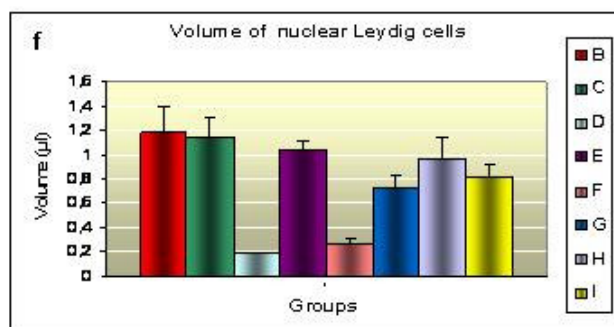
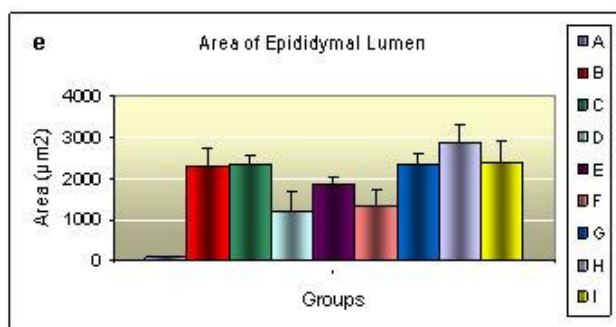
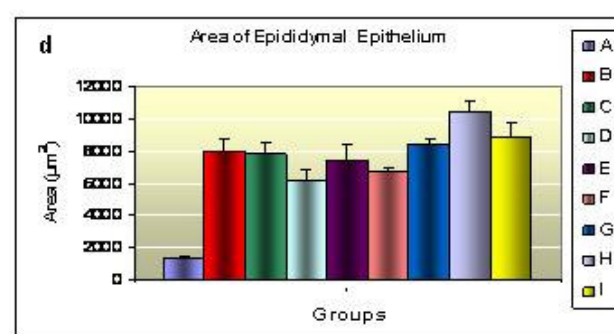
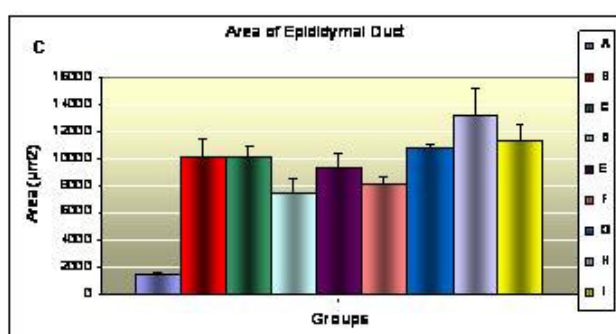
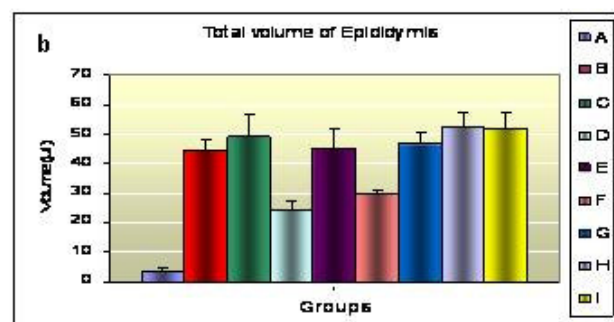
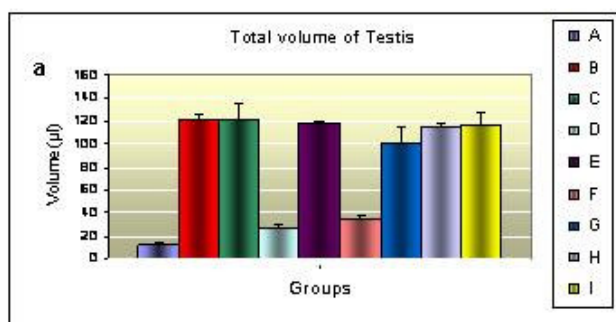
^b: Significantly different from values for cryptorchidic mice, p<0.05.

^c: Significantly equal to cryptorchidic mice, p<0.05.

^d: Significantly different from all values in control, cryptorchidic and orchidopexic mice, p<0.05.

*: Parameter not analyzed in this group, p<0.05.

Figure 2- Results of the stereological analyses (a - f): total volume of the testes (a) and epididymis (b), areas of the epididymal duct (c), epididymal epithelium (d), and epididymal lumen (e), and nuclear volume of Leydig cells (f): **A** (immature group); **B** (mature group- 105 days old); **C** (mature group- 195 days old); **D**- unilateral cryptorchidic mice, **E**- contralateral cryptorchidic mice and **F**- bilateral cryptorchidic mice; **G**- unilateral orchidopexic mice, **H**- contralateral orchidopexic mice, **I**- bilateral orchidopexic mice. Testosterone levels (g): **1**- (mature group- 105 days old); **2**- (mature group- 195 days old); **3**- unilateral cryptorchidic mice; **4**- bilateral cryptorchidic mice, **5**- unilateral orchidopexic mice and **6**- bilateral orchidopexic mice.



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IV. CONCLUSÕES

Este trabalho permite as seguintes conclusões:

(1) O criptorquidismo experimental realizado em animais com 15 dias pós natal mimetizou o criptorquidismo congênito, causando atrofia e significativas alterações estereológicas do testículo e epidídimo. Em relação aos controles, houve uma recuperação significativa dos parâmetros estereológicos (áreas e volumes do testículo e epidídimo) nos animais submetidos à orquidopexia.

(2) A dosagem de testosterona sérica não foi significativamente diferente em nenhum grupo analisado, contudo, houve uma redução significativa no número e no volume do núcleo das células de Leydig nos animais criptorquídeos e animais submetidos à orquidopexia. Apesar desta diferença, os níveis de testosterona sérica não acompanharam a variação ocorrida pelo número de células de Leydig, podendo este fato ser justificado por um mecanismo compensatório da atividade das células de Leydig.

(3) As análises mostraram que as alterações estruturais foram mais acentuadas no testículo do que no epidídimo, sugerindo maior sensibilidade do testículo ao aumento de temperatura. A orquidopexia é um método eficaz para restauração da espermatogênese e alterações histológicas da cabeça do epidídimo causadas pelo criptorquidismo.