UNIVERSIDADE ESTADUAL DE CAMPINAS

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"Análise quantitativa, duração do ciclo do epitélio seminífero e produção espermática do testículo do gerbilo da Mongólia (*Meriones unguiculatus*)"

Este exemplar corresponde à redação final da tese defendida pelo(a) candidato (a) Tânia Mara Segatelli
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Dedico,

Aos meus pais ***Tertuliano*** *e* ***Ivone***

"A vocês que me deram a vida e me ensinaram a vivê-la com dignidade, iluminaram os caminhos obscuros com afeto e dedicação para que caminhasse sem medo e cheia de esperança, doando-se por inteiros e renunciaram aos seus sonhos, para que muitas vezes pudesse realizar o meu. A vocês pais, por natureza, por afeto e amor, faço de minha conquista o instrumento de gratidão e reconhecimento por tudo quanto recebi de vocês, meus queridos pais".

Aos meus irmãos, ***Cláudio, Márcio e Aldo***

"Pela força, incentivo e confiança que depositaram em mim,

com muito carinho e amor..."

Ao ***Anderson,***

"Pelo companherismo, incentivo e ajuda,

com muito amor..."

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"Ser orientador não é coisa de um dia. São atos, palavras e atitudes, que solidificam no tempo e não se apagam mais, o que o torna especial. A você que soube transmitir seus conhecimentos, suas experiências e apoiar-me em minhas dificuldades, meu maior agradecimento e meu profundo respeito".

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SUMÁRIO

RESUMO

O gerbilo (*Meriones unguiculatus*) é um roedor nativo das regiões áridas da Mongólia e China, é de especial interesse em pesquisas biomédicas e tem sido criado em laboratório desde 1960. Estudos sobre a estrutura e função do testículo do gerbilo são raros, em especial sobre a espermatogênese. O presente trabalho é o primeiro a realizar uma investigação mais detalhada sobre a duração do ciclo espermatogênico e analisar morfométrica e funcionalmente o testículo do gerbilo. Através da técnica de marcação com ^{3}H-timidina, a duração do ciclo do epitélio seminífero foi estimada em 10,55 ± 1,0 dias e a duração total do processo espermatogênico foi de 47,47 dias, considerando que 4,5 ciclos são necessários para completar o processo. Os compartimentos tubular e intersticial ocuparam volume de 92% e 8%, respectivamente. Baseado no volume ocupado pelo compartimento tubular no parênquima testicular e no diâmetro do túbulo seminífero, aproximadamente 10 metros de túbulos seminíferos foram encontrados por testículo e 18 metros por grama de testículo no gerbilo. Cerca de 12 espermatócitos primários foram formados para cada espermatogônia do tipo A1. O índice meiótico observado foi de 2,8, representando 30% de perdas celulares durante as divisões meióticas. O número de células de Sertoli por grama de testículo foi de 28 milhões, com capacidade de suporte individual de 21 células germinativas. A produção espermática diária por grama de testículo foi de 33 milhões, sugerindo alta eficiência espermatogênica nessa espécie. O gerbilo pode ser utilizado como modelo para futuras investigações do processo espermatogênico.

ABSTRACT

The gerbil *(Meriones unguiculatus)* is a rodent native of the arid regions of Mongolia and China, is of special interest in biomedical research and has been bred in laboratory since 1960. Studies on the structure and function of the gerbil testis are sporadic and, moreover those on spermatogenesis are rare. This is the first study to perform a more detailed investigation about duration of the spermatogenic cycle and morphometric and functional analysis of the gerbil testis. Through ^{3}H-thymidine injection tecnic, the mean duration of seminiferous epithelium cycle was determined to be 10.55 ± 1.0 days and the total duration of spermatogenesis based on 4.5 cycles was 47.47 days. The volume density of tubular and interstitial compartments was approximately 92% and 8%, respectively. Based on the volume of the testis parenchyma and the volume occupied by seminiferous tubules in the testis and the tubular diameter, about 9 and 18 meters of seminiferous tubules were found per testis and per gram of testis, respectively. About 12 primary spermatocytes were formed for each type A1 spermatogonia. The meiotic index was 2.8. This result shows that 30% of cell loss occurs during the two meiotic divisions. The number of Sertoli cell per gram of the testis was 28 million and the supporting capacity was 21 germ cell. The daily sperm production per gram of the testis was 33 million. This value suggest high spermatogenic efficiency in this specie. The gerbil could be utilized as an animal model for future investigation of spermatogenic process.

INTRODUÇÃO GERAL

O testículo de mamíferos é um órgão complexo com funções endócrina e exócrina. Apresenta-se revestido por cápsula de tecido conjuntivo, a túnica albugínea que envia septos para o interior do órgão dividindo-o em lóbulos e formando o mediastino testicular. Morfofuncionalmente apresenta dois compartimentos: (i) intersticial ou intertubular, contendo células e fibras do tecido conjuntivo, vasos sanguíneo e linfático e células de Leydig, principal fonte de andrógeno do organismo; (ii) tubular, formado por túbulos seminíferos, no interior dos quais ocorre a espermatogênese (formação do gameta masculino). Apesar de o testículo dos mamíferos apresentar estrutura padrão para as diversas espécies, há grande variação em relação à proporção volumétrica dos seus diversos componentes, principalmente em relação aos túbulos seminíferos, células de Leydig e disposição e proporção dos vasos e espaços linfáticos (RUSSELL *et al.*, 1990).

A maior parte do parênquima testicular apresenta-se constituído pelo compartimento tubular, ocupando cerca de 70 a 90% na maioria das espécies de mamíferos estudadas (FRANÇA & RUSSELL, 1998). Os túbulos seminíferos são constituídos, de sua porção externa para interna, de túnica própria, epitélio seminífero e luz. O epitélio seminífero apresenta-se constituído por diferentes gerações de células germinativas (as espermatogônias, os espermatócitos e as espermátides em várias fases de desenvolvimento) e por células somáticas, as células de Sertoli. As espermatogônias podem ser classificadas em duas categorias: (i) indiferenciadas, compreendendo as espermatogônias isoladas (Ais), pareadas (Apr) e alinhadas (Aal). As espermatogônias isoladas podem dar origem tanto as pareadas quanto as tronco (de ROOIJ, 2001), e (ii) diferenciadas, compreendendo as espermatogônias do tipo A (A1-4), dependendo da espécie, intermediária (In) e B, comprometidas irreversivelmente com a formação dos espermatozóides (de ROOIJ, 1998). Existem consideráveis diferenças em relação ao número de gerações de espermatogônias diferenciadas nas diversas espécies de mamíferos. No entanto, o número geralmente não ultrapassa seis gerações (RUSSELL *et al.*, 1990; FRANÇA & RUSSELL, 1998; de ROOIJ, 1998; de ROOIJ & GROOTEGOED, 1998; de ROOIJ, 2001).

As células germinativas não estão distribuídas ao acaso no interior dos túbulos seminíferos, mas organizadas em associações celulares ou estágios ordenados de modo espécie-específicos. O conjunto completo de estágios, organizados em seqüência progressiva do desenvolvimento das células germinativas em um dado segmento do túbulo seminífero, é denominado ciclo do epitélio seminífero (CES) (LEBLOND & CLERMONT, 1952; CLERMONT, 1972; RUSSELL *et al.*, 1990). O padrão cíclico é marcado por evolução progressiva e sincronizada de células espermatogênicas individuais e translocação concomitante de células em maturação do compartimento basal para o adluminal do túbulo seminífero. O ciclo celular é rigidamente controlado, existindo associações celulares ou estágios precisos e relativamente constantes para cada espécie. Mapas dos estágios do CES podem ser preparados para cada espécie com a finalidade de caracterizar as associações celulares apresentadas em secções histológicas transversais do túbulo seminífero (RUSSELL *et al.*, 1990; WEINBAUER *et al.*, 1998; 2001). O número de associações celulares é variável dependendo da espécie e do método de identificação dos estágios do ciclo. Basicamente dois critérios de identificação dos estágios são utilizados, o método do sistema acrossômico, baseado na formação e desenvolvimento do sistema acrossômico das espermátides em maturação, onde os estágios são arbitrariamente designados e o número de estágio para cada espécie é variável (RUSSELL *et al.*, 1990) e o método da morfologia tubular, baseado na forma e na localização dos núcleos das espermátides e na presença de figuras de divisões meióticas, permitindo a identificação de oito estágios do ciclo para todas as espécies (BERNSDTSON, 1977; ORTAVANT *et al.*, 1977; GUERRA, 1983). A freqüência de aparecimento de cada estágio durante o CES é variável, espécie-específica e diretamente proporcional a duração de cada estágio. Portanto, o estágio de duração mais curta aparece menos freqüentemente do que o estágio de duração mais longa (CLERMONT & ANTAR, 1973; HESS *et al.*, 1990; SAIDAPUR & KAMATH, 1994).

O intervalo de tempo necessário para que uma série completa de estágios apareça em um determinado ponto dentro do túbulo seminífero é denominado de duração do CES (LEBLOND & CLERMONT, 1952), que é considerada uma constante biológica espécie-específica, não sendo alterada por fatores naturais, manipulações experimentais e pela idade (CLERMONT & HARVEY, 1965; RUSSELL *et al.*, 1983). No entanto, podem ocorrer

variações significativas entre raças ou linhagens diferentes dentro de uma mesma espécie (RUSSELL *et al.*, 1990; van HAASTER & de ROOIJ, 1993). Utilizando-se a técnica de transplante de espermatogônias de ratos para camundongos, observou-se que a duração da espermatogênese está sob controle do genótipo das células germinativas (FRANÇA *et al.*, 1998). Embora a duração do CES possa ser determinada com um certo grau de acurácia, o valor é aproximado devido à dificuldade em se determinar o ponto exato no qual a espermatogônia do tipo A inicia o processo (SWIERSTRA & FOOTE, 1965; CLERMONT, 1972; FRANÇA & RUSSELL, 1998). O número de ciclos necessários para o processo completo da espermatogênese depende da espécie e o local de escolha como ponto inicial do processo (SWIERSTRA & FOOTE, 1965; SWIERSTRA, 1968). Em média, 4,5 ciclos são necessários para a espermatogônia diferenciada do tipo A1 se transformar em espermatozóide (AMANN & SCHANBACHER, 1983; CLERMONT, 1972; RUSSELL *et al.*, 1990).

A partir do conhecimento da duração do CES, pode-se estimar a duração de cada estágio, das várias fases de desenvolvimento da espermatogênese (prófase meiótica, divisões meióticas e espermiogênese), do processo espermatogênico como um todo, além de permitir avaliar os efeitos da irradiação e das drogas sobre as células germinativas, embasando estudos sobre a cinética espermatogonial (de ROOIJ *et al.*, 1986). Por outro lado, é importante salientar que o conhecimento da duração do CES constitui requisito básico para se estimar a produção espermática diária (PED), parâmetro usado na avaliação da capacidade espermatogênica em machos de qualquer espécie, raça ou linhagem (FRANÇA, 1992; CASTRO *et al.*, 1997).

A duração da espermatogênese tem sido determinada em mamíferos utilizando-se várias técnicas, como injeção de colchicina (ROOSEN-RUNGE, 1951), irradiação (OAKBERG, 1956), elevação da temperatura do testículo (ASDELL & SALESBURY, 1941), injeção com isótopos (ORTAVANT, 1956; HELLER & CLERMONT, 1963; SWIERSTRA & FOOTE, 1965; FOOTE *et al.*,1972; BARR, 1973; CLERMONT & ANTAR, 1973; SMITHWICK *et al.*, 1996) e injeção com 5-bromodioxiuridina (TOOLEN, 1990; ROSIEPEN *et al.*, 1994; DOLBEARE, 1995; ROSIEPEN *et al.*, 1995; ROSIEPEN *et al.*, 1997). No entanto, o método disponível mais utilizado é a injeção de isótopo

utilizando-se a Timidina-^{3}H, radioisótopo específico que se liga ao ácido desoxiribonucléico (DNA). Após a injeção de timidina, as células que estão em fase de síntese de DNA são marcadas (CLERMONT *et al.*, 1959). No epitélio seminífero, localizam-se os núcleos das espermatogônias, preparando-se para a mitose e dos espermatócitos preparando-se para a meiose, além de permitir alta resolução radiográfica e ser esgotada em curto período de tempo após administração (SWIERSTRA & FOOTE, 1965; BARR, 1973). Coletando-se amostras de testículo em diferentes intervalos de tempo após a administração da Timidina-^{3}H, é possível determinar o tempo de desenvolvimento das células germinativas marcadas (CLERMONT *et al.*, 1959; HELLER & CLERMONT, 1963; CLERMONT & HARVEY, 1965).

Aplicando-se métodos estereológicos em análises do testículo de mamíferos, importantes informações têm sido obtidas para as células germinativas (ROOSEN-RUNGE, 1955; CLERMONT & PEREY, 1957; RUSSELL & CLERMONT, 1977; WING & CHRISTENSEN, 1982; RUSSELL & PETERSON, 1984, FRANÇA *et al.*, 1995), as células de Sertoli (BUSTOS-OBREGON, 1970; CAVICCHIA & DYM, 1977, FRANÇA *et al.*, 1993) e as células de Leydig (MORI & CHRISTENSEN, 1980). O conhecimento do ciclo do epitélio seminífero, a caracterização dos estágios que o compõem, a determinação da freqüência desses estágios, bem como o cálculo da duração dos eventos espermatogênicos, são parâmetros fundamentais na quantificação da espermatogênese (BERNDTSON, 1977). A morfometria auxilia o entendimento do processo espermatogênico principalmente quando correlacionado a dados bioquímicos e fisiológicos, é essencial no estabelecimento do padrão de divisão e renovação das espermatogônias e permite estimar a eficiência da espermatogênese em qualquer de suas etapas. Tal eficiência é traduzida pelas razões encontradas entre os números celulares obtidos (WING & CHRISTENSEN, 1982; GAYTAN *et al.*, 1986; CASTRO *et al.*, 1997).

Durante a espermatogênese, as células germinativas e as células de Sertoli interagem de maneira complexa, tanto física como fisiologicamente (LEBLOND & CLERMONT, 1952; CLERMONT, 1972; RUSSELL *et al.*, 1990). Estudos mostram que o número de células de Sertoli por testículo é o principal fator na determinação da produção espermática e no tamanho do testículo (ORTH *et al.*, 1988; HESS *et al.*, 1993; FRANÇA *et*

al., 1995), baseado no fato de que as células de Sertoli têm capacidade de suporte de células germinativas relativamente fixas para cada espécie e que a população deste tipo celular se mantém estável após a puberdade (STEINBERGER & STEINBERGER, 1971; ORTH, 1982; ORTH *et al.*, 1988; FRANÇA & RUSSELL, 1998). Desta forma, o número de células germinativas suportadas por uma única célula de Sertoli, principalmente o número de espermátides, é o melhor indicativo da eficiência funcional das células de Sertoli e da produção espermática (RUSSELL & PETERSON, 1984; SINHÁ-HIKIM *et al.*, 1989; FRANÇA & RUSSELL, 1998). Levando se em conta que a população de células de Sertoli é estável ao longo dos estágios do ciclo, este tipo celular tem sido utilizado numericamente como ponto de referência para a quantificação e correção de contagens de células germinativas (CLERMONT & MORGENTALER, 1955; BUSTOS-OBREGON, 1970; SKAKKEBAEK & HELLER, 1973). Assim, os índices de células de Sertoli, que correspondem às razões entre o número de células germinativas e o número de células de Sertoli, propiciam base para os estudos das alterações no processo espermatogênico em decorrência de condições patológicas e terapêuticas (RUSSELL & CLERMONT, 1977; SINHÁ-HIKIM *et al.*, 1985; RUSSELL *et al.*, 1990).

A produção espermática diária por grama de testículo é eficaz na medida da eficiência espermatogênica, sendo útil em estudos comparativos. Vários métodos têm sido utilizados na avaliação da produção espermática diária: i) citometria (estimativa da reserva espermática dos testículos (Amann, 1970), ii) quantificação de espermatozóides ejaculados (Berndtson, 1977), e iii) morfometria do comprimento total dos túbulos seminíferos por testículo, do número total de células de Sertoli por testículo, razão entre o número de espermátides arredondadas por células de Sertoli e freqüência e duração de determinado estágio do ciclo, dependentes da espécie (FRANÇA, 1992).

A espermatogênese é um dos processos celulares mais produtivos de auto-renovação não sendo observado em outros tecidos. A taxa entre auto-renovação e diferenciação de células-tronco espermatogoniais deve ser 1,0. Se houver desequilíbrio entre auto-renovação e diferenciação, poderá reduzir o epitélio seminífero a células-tronco e um tumor pode ser formado (de ROOIJ, 2001). Em ratos, uma única célula-tronco é capaz de produzir 4096 espermatozóides, entretanto, a morte celular durante o processo reduz a

produção final substancialmente (RUSSELL *et al.*, 1990). Em mamíferos, a apoptose de células germinativas é parte integral da espermatogênese, ocorrendo normalmente em várias fases do desenvolvimento (BLANCO-RODRÍGUEZ & MARTÍNEZ-GARCIA, 1996). A apoptose representa papel importante na homeostasia da espermatogênese, refletindo diretamente na produção espermática diária, característica de cada espécie (CLERMONT, 1972; RUSSELL *et al.*, 1990). Além de ocorrer normalmente, a apoptose pode ser induzida pela remoção do suporte hormonal, de fatores de crescimento ou por estímulos regulatórios, estresse ao calor, toxinas testiculares, agentes quimioterápicos e danos cromossomais (SINHA-HIKIM & SWERDLOF, 1999).

O gerbilo vem ganhando importância como modelo animal utilizado em pesquisas biomédicas e tem sido reproduzido em laboratório nos últimos anos (NAKAI *et al.*, 1960; SCHWENTKER, 1963; MARSTON & CHANG, 1965; RICH, 1968; NORRIS & ADAMS, 1981a,b; NORRIS & RALL, 1983; BARFIELD & BEEMAN, 1986; CLARK *et al.*, 1986; KRESS *et al.*, 1989; KRESS & MARDI, 1990). É um roedor originário das regiões da Mongólia e nordeste da China. Pertence a família Cricetidae, subfamília Gerbilinae, gênero Meriones sp., espécie *Meriones unguiculatus*. Poucos estudos relatam a biologia reprodutiva dos gerbilos, inclusive a estrutura e a função testiculares. Trabalhos sobre a morfologia básica do testículo do gerbilo vêm sendo realizados, incluindo a descrição do desenvolvimento pós-natal e da hiperplasia testicular (NINOMIYA & NAKAMURA, 1987 a, b), a ultra-estrutura do acrossoma e a cinética da espermatogênese (SEGATELLI *et al.*, 2000, 2002). Dessa forma, estudos mais detalhados da estrutura e função do testículo do gerbilo se fazem necessários. Os objetivos do presente trabalho foram:

- calcular as proporções volumétricas (%) entre os diferentes componentes do parênquima testicular;
- determinar o comprimento total dos túbulos seminíferos;
- estimar a duração do ciclo do epitélio seminífero em dias;
- determinar a população celular em secções transversais de túbulos seminíferos no estágio VII do ciclo;

- determinar razões quantitativas entre células da linhagem espermatogênica e células de Sertoli a partir das contagens obtidas no estágio VII;
- calcular o número de células de Sertoli por testículo e por grama de testículo;
- estimar a produção espermática diária por testículo e por grama de testículo e;
- determinar o volume das células de Leydig e o número de células por testículo e por grama de testículo.

CAPÍTULO I

ARTIGO SUBMETIDO À REVISTA *JOURNAL OF MORPHOLOGY*

Duration of the spermatogenesis and morphometry of the testis in gerbil (*Meriones unguiculatus*)

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Key words: Meriones unguiculatus; cycle length; thymidine; spermatogenesis

Duration of the spermatogenesis and morphometry of the testis in gerbil (*Meriones unguiculatus*)

Abstract

The gerbil *(Meriones unguiculatus)* is a rodent native of the arid regions of Mongolia and China, is of special interest in biomedical research and has been bred in laboratory since 1960. Studies on the structure and function of the gerbil testis are sporadic and, moreover those on spermatogenesis are rare. This is the first study to perform a more detailed investigation about of duration of the spermatogenic cycle and morphometric and functional analysis of the gerbil testis. Through ^{3}H-thymidine injection technique, the mean duration of seminiferous epithelium cycle was determined to be 10.55 ± 1.0 days and the total duration of spermatogenesis based on 4.5 cycles was 47.47 days. The volume density of tubular and interstitial compartments was approximately 92% and 8%, respectively. Based on the volume of the testis parenchyma and the volume occupied by seminiferous tubules in the testis and the tubular diameter, about 9 and 18 meters of seminiferous tubules were found per testis and per gram of testis, respectively. About 12 primary spermatocytes were formed from each type A1 spermatogonia. The meiotic index was 2.8. This result shows that 30% of cell loss occurs during the two meiotic divisions. The number of Sertoli cell per gram of the testis was 28 million and the supporting capacity was 21 germ cell. The daily sperm production per gram of the testis was 33 million. This value suggest high spermatogenic efficiency in this specie. The gerbil could be utilized as an animal model for future investigation of spermatogenic process.

Introduction

The spermatogenic process is a cyclic, highly organized and coordinated event in which diploid spermatogonia differentiate into mature haploid spermatozoa (Russell *et al.* 1990a). The complexity of this process necessitates tight and well-balanced control mechanisms evidenced by the precise duration of spermatogenesis and the presence of defined germ-cell associations. These germ-cell associations are referred to as stages of spermatogenesis, and they vary in number and duration in a species-specific manner (Weinbauer *et al.* 2001). The sequence of events that occurs from the disappearance of a given cellular association to its reappearance in a given area of the seminiferous epithelium constitutes the seminiferous epithelium cycle (SEC) (Leblond and Clermont, 1952). The time interval required for one complete series of cellular associations to appear at one point within the tubule is called duration of the SEC. The total duration of spermatogenesis, that takes approximately 4.5 cycles, lasts from 30 to 75 days in mammals (França and Russell, 1998). Although strain or breed difference can be found in the literature among members of the same species (Russell *et al.*, 1990a), the duration of the spermatogenic cycle has been generally considered to be constant for a given species. Recent work has demonstrated that the spermatogenic cycle duration is under the control of germ cell genotype (França *et al.*, 1998).

Estimates for duration of the seminiferous epithelium cycle (SEC) have been obtained by various methods (Roosen-Runge, 1951; Clermont and Perey, 1957; Clermont and Antar, 1973; Rosiepen *et al.* 1994; Dolbeare, 1995; Smithwick *et al.* 1996a, b; Rosiepen *et al.* 1997; Weinbauer *et al.* 1998; França *et al.*, 1999). The most utilized method to determine the duration of spermatogenesis is the isotope technique employing thymidine, a specific precursor for deoxyribonucleic acid (DNA) (Swierstra and Foote, 1965; Swierstra, 1968; Barr, 1973).

Most quantitative investigation of spermatogenesis required identification of the stages of seminiferous epithelium cycle and the knowledge of its duration (Berndtson, 1977). Numerous quantitative studies have been made on germ cells (Roosen-Runge, 1955; Clermont and Perey, 1957; França *et al.* 1995), Sertoli cells (Bustos-Obregón, 1970;

Cavicchia and Dym, 1977; Russell and Peterson, 1984) and Leydig cells (Mori and Christensen, 1980; Rey *et al.* 1996) in mammals. The number of Sertoli cells is important since it is related to the spermatogonial number, spermatid number, and spermatozoa production rates in various species. The ratio between germ and Sertoli cells (Sertoli cell index) is the best available parameter that shows the functional activity of somatic cells (Johnson, 1995). It also allows, with morphometric and kinetic data, estimation of the daily sperm production (DSP). Accurate morphometric information can provide answers to important questions about the spermatogenic process, and also is valuable for correlation with physiological and biochemical findings in order to develop a more complete understanding of spermatogenesis (Wing and Christensen, 1982; Gaytan *et al.* 1986).

The gerbil (*Meriones unguiculatus*) is a rodent native to the arid regions of Mongolia and China and is of special interest in biomedical research and it has been bred in the laboratory since 1960. Little is known about its reproductive biology (Schwentker, 1963; Rich, 1968; Williams, 1974). Studies on the basic morphology are sporadic and, moreover, those include descriptions of the postnatal development and benign testicular hiperplasia (Ninomiya and Nakamura, 1987,a,b), ultrastructural study of acrosome formation and kinetics of spermatogenesis (Segatelli *et al.*, 2000, 2002). The objective of the present study were to perform a detailed quantitative analysis of the testis, to estimate the duration of spermatogenesis and to estimate the daily sperm production in the gerbil, providing the necessary foundation for future studies of the reproductive cycle in this rodent.

Material and Methods

Animals

Sixteen adult male gerbils (*Meriones unguiculatus*), aged 3 months and weighing 65-80g were used. These animals were divided into two groups: eight to measure the duration of the seminiferous epithelium cycle and eight for morphometric analysis. The animals were maintained under controlled temperature (23 °C) and contolled lighting conditions (12:12h light-dark photoperiod, lights-out at 07:00). The experimental protocol followed the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation.

Thymidine injections and tissue preparation

To estimate the duration of spermatogenesis, the animals received intraperitoneally injections of tritiated thymidine (Thymidine [methyl – ^{3}H], specific activity 82.0 Ci/mmol, Amersham, Life Science, , England). The injections of 100μCi of ^{3}H-thymidine were performed using a hypodermic needle. Two animals were utilized for each time interval considered (1 hour, 7, 14 and 21 days) after thymidine injections. At the programmed time, the animals were anesthetized with ether and perfused with Karnovsky fluid (Sprando, 1990). Historesin-embedded tissues were sectioned at 3μm thickness and placed on glass slides. To perform autoradiographic analysis, the histological slides were dipped in autoradiography emulsion (Kodak NTB-2, Eastman Kodak Company, NY, USA) at 45°C. After drying for approximately 1h at 25 °C, sections were placed in sealed black boxes containing silica gel as a drying agent and stored in a refrigerator at 4°C for aproximately 30 days. Subsequently, testis sections were developed in Kodak D-19 solution at 15°C according to Bundy (1995), and stained with toluidine blue. Analyses of these sections were performed by light microscopy. Cells were considered labeled when 4-5 or more grain were present over the nucleus in the presence of low-to-moderate background.

Stages characterization and relative frequency

Stages of seminiferous epithelium cycle were characterized based on development of the acrosomic system and morphology of developing spermatids. This method provided twelve stages of the SEC. The relative stage frequencies were determined from the analysis of 200 seminiferous tubule cross-sections in 12 animals at 400X magnification, according Hess *et al* (1990). Both stages characterization and relative frequencies were described previously for the gerbil (Segatelli *et al.* 2000; 2002). The duration of the seminiferous epithelium cycle was estimated by considering the most advanced germ cell type labeled at each period after the isotope injection and based on the stages frequencies.

Morphometric and stereological analysis of the testis

Eight animals were weighted, anesthetized with ether and perfused with Karnovsky fluid (Sprando, 1990). Testis were collected, weighted and was determined the percentage occupied by tunica albuginea in parenchyma testicular. In present work the mediastinum was not considered because its very small. Testis samples were embedded in Historesin®, sectioned at 4μm thickness, placed on glass slides and stained with toluidine blue and Schiff-hematoxylin periodic acid. The tubular diameter of the seminiferous tubule and the height of the seminiferous epithelium were measured at 100X magnification using an ocular micrometer calibrated with a stage micrometer. Thirty tubular profiles that were round or nearly round were chosen randomly and measured for each animal. The epithelium height was obtained in the same tubule sections utilized to determine tubular diameter. The volume densities of various testicular tissue components were determined by light microscopy using a 441-intersection grid placed in the ocular of the light microscope. Twenty fields chosen randomly (8820 points) were scored for each animal at 400X magnification. Points were classified as one of the following: seminiferous tubule, comprising tunica propria, epithelium and lumen; Leydig cell; blood and lymphatic vessels; connective tissue; and others (including fibers and cells of conective tissue). The volume of each component of the testis was determined as the product of the volume density and testis volume. To obtain a more precise measure of testis volume, 3.15% of testicular capsule was excluded from testis weight. The total length of seminiferous tubule per testis and per gram

of testis, expressed in meters, was obtained by dividing the seminiferous tubule volume by the squared radius of the tubule times the pi value (πR^2) (Johnson and Neaves, 1981)

Cell counts and cell numbers

All germ cell nuclei and Sertoli cell nucleoli present at stage VII of the SEC were counted in 10 round or nearly round seminiferous tubule cross- sections, chosen at random, for each animal. These counts were corrected for section thickness and nucleus or nucleolus diameter according to Abercrombie (1946), modified by Amann (1962). The following germ cells were counted: type A1 spermatogonia, leptotene primary spermatocytes, pachytene primary spermatocytes and round spermatids. The following ratios were obtained from the corrected counts:

- pachytene spermatocytes / type A1 spermatogonia, to estimate the eficiency coeficient of spermatogonial mitosis;
- round spermatids / type A1 spermatogonia, to obtain the geral rendiment of spermatogenesis;
- round spermatids / pachytene spermatocytes, to obtain the meiotic index;
- round spermatids / Sertoli cell nucleoli, to estimate the Sertoli cell index;
- total number of germ cells / Sertoli cell nucleoli, to obtain the total capacity of suport of the Sertoli cell.

The total number of Sertoli cells was determined from the corrected counts of Sertoli cell nucleoli per seminiferous tubule cross-section and the total length of seminiferous tubules according to the method described by Hochereau-de Reviers and Lincoln (1978). The daily sperm production (DSP) per testis and per gram of testis were obtained according to the following formula developed by França (1992): DSP = total number of Sertoli cells per testis x the ratio of round spermatids to Sertoli cells at stage VII x stage VII relative frequency (%) / stage VII duration (days).

Individual volume of a Leydig cell was obtained from nucleus volume and the proportion between nucleus and cytoplasm. The diameters of thirty nuclei showing evident nucleolus and apherical form were measured for each animal. Leydig cell nuclear volumes was expressed μm^3 and obtained by the formula $(4/3)\pi R^3$, were R = nuclear diameter/2. To

calculate the proportion between nucleus and cytoplasm, a 441-point square lattice was placed over the sectioned material at 400X magnification. One thousand points over Leydig cells were counted for each animal. The number of Leydig cells per testis and per gram of testis were estimated from the Leydig cell individual volume and the volume occupied by Leydig cells in the testis parenchyma.

Statistical Analysis

Data were analyzed by Descriptive Statistic. Details of the methods used are found in Banzatto & Kronka (1989).

Results

Stages of the seminiferous epithelium cycle and relative stage frequencies

Based on the development of the acrosomic system and changes in the nuclear morphology, XII stages or cellular associations were characterized in the gerbil (Segatelli *et al.*, 2000, 2002). The composition of each stage can be seen in Figure 1. The relative frequency of each stage is shown in Figure 2. Stage I showed the highest (13.8%), while stage V showed the lowest frequency (4%).

Duration of spermatogenesis

The most advanced labeled germ cell type present at several times after the ^{3}H-thymidine injections (1 hour, 7, 14, and 21 days) are shown in Table 1 and Figure 2. Approximately one hour after injection, the most advanced labeled germ cell type were leptotene primary spermatocytes present at stage VII (Fig. 3a). Other labeled germ-cell nuclei types found in remaining stages presented the typical morphological characteristics of type A, intermediate and type B spermatogonia. The most advanced labeled germ cell type 7 days after injection were the pachytene spermatocytes, observed at stage III (Fig. 3b). At the middle of stage XII, meiotic figures from first meiotic division were observed as the most advanced labeled germ cell type 14 days after injection (Fig. 3c). The most advanced germ cell type labeled 21 days after injection were the round spermatid at stage VI of the cycle (Fig. 3d). The cycle duration was estimated taking into account the most advanced labeled germ cell type for each time period considered after thymidine injections and the stages frequencies. The mean duration of the SEC was estimated to be 10.55 ± 1.0 days. This mean value was obtained based on labeling of leptotene spermatocytes and intermediate labeling points for the various time intervals considered (Table 1). The duration of each stage of the SEC was determined taking into account the cycle duration and the percentage of occurrence of each stage (Fig. 2). The stage I was the longest stage (1.46 days), while the shortest was stage V (0.42 days). Considering that aproximately 4.5 cycles are necessary for the spermatogenesis process to be completed, the total duration of spermatogenesis was estimated as being 47.47 days.

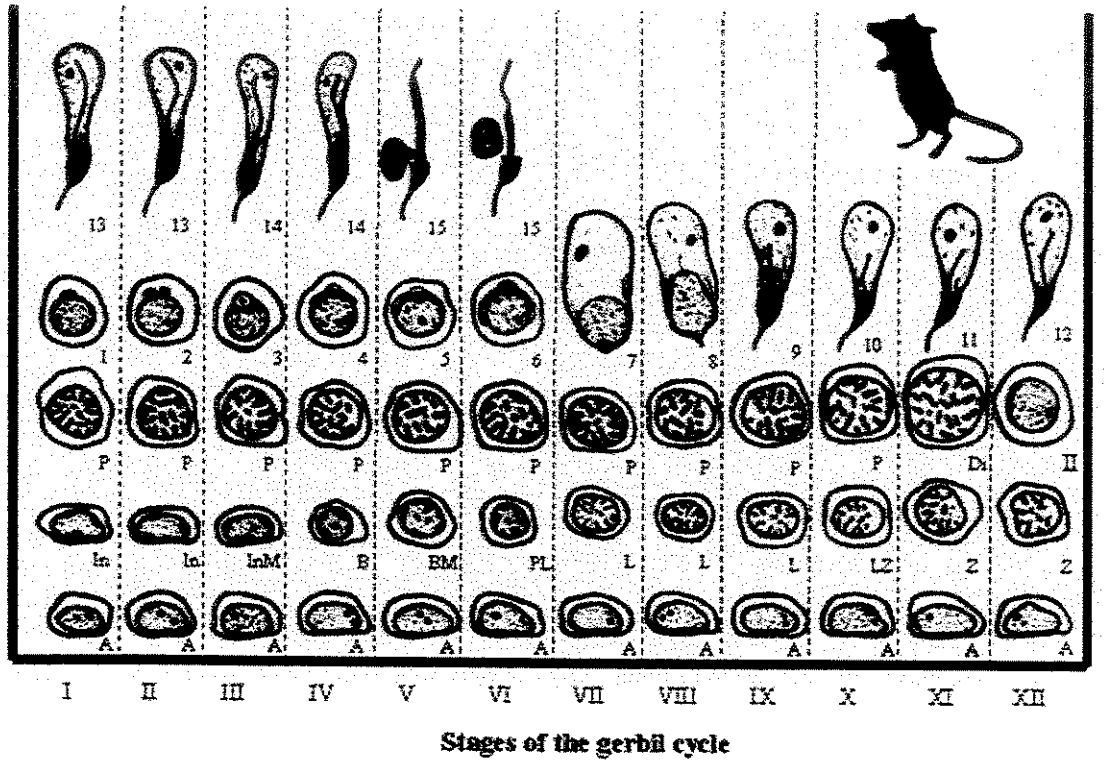


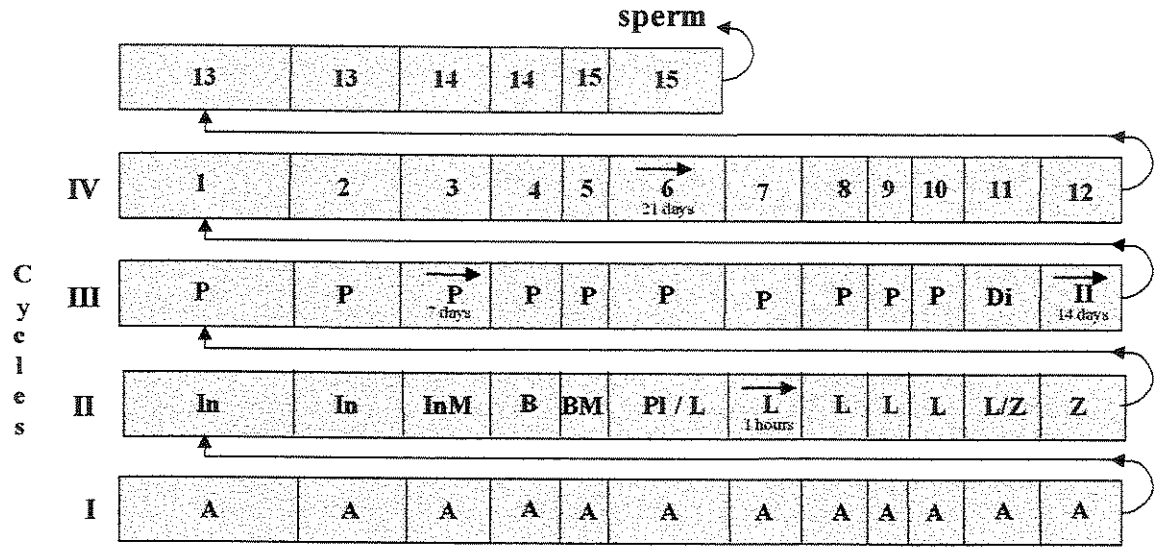
Figure 1. A map of the seminiferous epithelium cycle of the gerbil. The vertical columns, designated by roman numerals, depict cell associations. The developmental progression of a cell is followed horizontally until the right hand border of the cycle map is reached. The cell progression continues at the left of the cycle map one row up. The cycle map ends with the completion of spermiation. The symbols used designate specific phases of cell development types: A (A), Intermediate (In), and B (B) spermatogonial cells, mitotic divisions (InM) and (BM); meiotic phase: preleptotene (Pl), leptotene (L), zygotene (Z), pachytene (P), diplotene primary spermatocyte (Di) and secondary spermatocyte (II). The cardinal numerals: spermiogenesis phases (Segatelli *et al.*, 2000, 2002).

Table 1: The length (days) of seminiferous epithelium cycle (X ± SEM).

Time after injection	**Most advanced germ cell type labeled**	**Stage of the cycle**	**Number of cycles traversed**	**Cycle length based on labeling in leptotene**	**Cycle length based on intermediate labeling point**
89 minutes	Leptotene	VII	–	–	–
6.96 days*	Pachytene	III	0.77	9.00	10.96
13.98 days*	Meiotic figures	XII	1.41	9.89	11.33
20.98 days*	Round spermatid	VI	2.01	10.43	11.72
Mean[1]				9.77	11.33

* total time after thymidine injection minus 89 minutes.

[1] Mean duration of the cycle based on labeling in leptotene and on intermediated labeling points. X = 10.55 ± 1.0 days



Stages	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Frequency (%)	13.8	10.12	8.16	7.87	4,0	11.25	7,54	7.16	5.9	7.62	8.12	8.95
Duration (days)	1.46	1.06	0.86	0.83	0.42	1.18	0.79	0.75	0.62	0.80	0,85	0,94

Figure 2. Diagram showing the germ cell composition, the frequencies (%), and the duration in days for each stage of the seminiferous epithelium cycle. Also depicted is the most advanced germ cell type labeled at the twelve stage of the cycle at different time periods (1h, 7, 14, and 21 days) following ^{3}H-thymidine injections (arrows). The roman vertical numbers indicate the spermatogenic cycles and the horizontal roman numbers the stages of cycle with their relative frequencies (%) and durations (days). The space given to each stage is proportional to its frequency and duration. The letters within each column indicate the germ cell types present at each stage of the cycle. Type-A (A), In (In) and B (B) spermatogonia, mitotic divisions (InM) and (BM); preleptotene (Pl), leptotene (L), zygotene (Z), pachytene (P), diplotene (Di), spermatocytes; secondary spermatocytes (II); round (1-6) and elongated (7-15) spermatids.

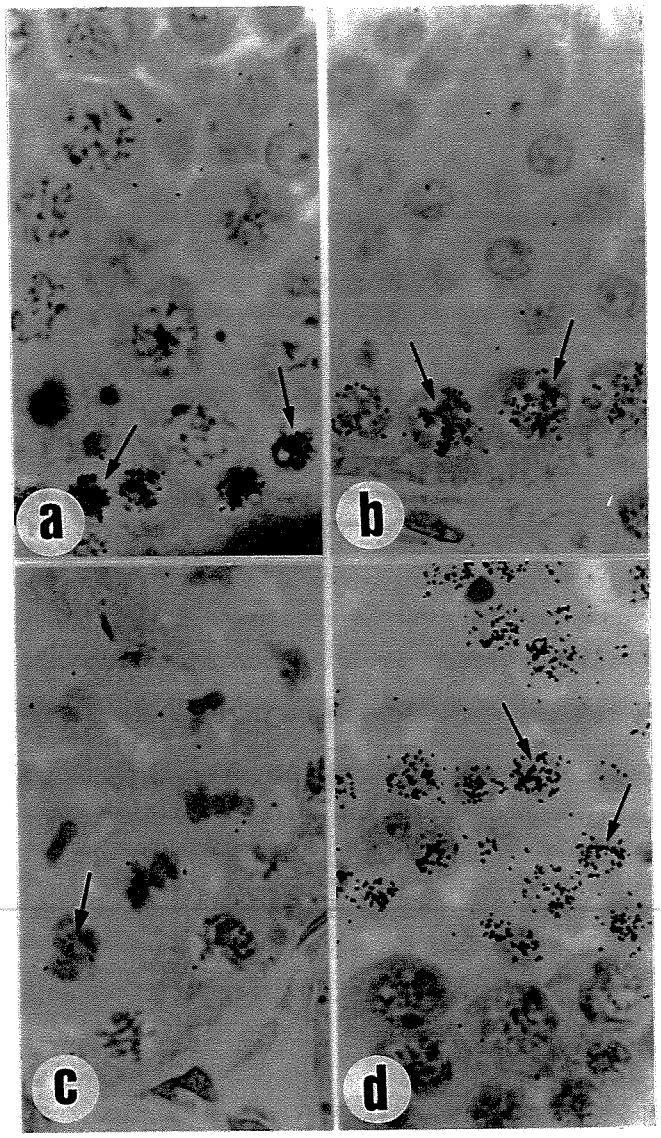


Figure. 3. Most advanced labeled germ cell type found after different time periods following intraperitoneally injections of ^{3}H-thymidine. (a) Approximately one hour after injection, leptotene primary spermatocytes (arrows) at stage VII. (b) Seven days after injection, pachytene spermatocytes (arrows), observed at stage III. (c) Fourteen days after injection, meiotic figures from first meiotic divisions present at the midlle of stage XII (arrow). (d) Twenty one days after injection, round spermatids (arrows), at stage VI. Toluidine blue and X1000 magnification.

Morphometry of the testis

The mean testis weight found for the gerbil was approximately 0.54g, providing a gonadosomatic index (testes mass divided by body weight) of approximately 0.72%. The volume density of tubular and interstitial compartment was approximately 92% and 8%, respectively (Table 2). The mean tubular diameter and epithelium height were 253 and 100μm, respectively. Based on volume of the testis parenchyma (testis weigh minus tunica albuginea weight) and the volume occupied by seminiferous tubules in the testis and the tubular diameter, about 10 meters of seminiferous tubules were found per testis and 18 meters per testis gram (Table 2). The table 3 shows the corrected number of germ cell types and Sertoli cell nucleoli per section of seminiferous tubules in stage VII of SEC. Cell ratios were obtained from the corrected counts (Table 4). About 12 primary spermatocytes were found per each type A1 spermatogonia present at stage VII of the cycle. The meiotic index, measured as the number of round spermatids produced per pachytene spermatocytes, was 2.8. This results shows that 30% of cell loss occurs during the two meiotic divisions. The overall spermatogenic yield was 33. The Sertoli cell efficiency, estimated from the total number of germ cells, and the number of round spermatids per Sertoli cell was de 21 and 12.6, respectively (Table 4). Sertoli cell number per testis was 15 million. The daily sperm production per testis and per gram of testis was 18 and 33 million, respectively (Table 5). Leydig cell nuclear volume and cell size were approximately 328 and 1148μm^3, respectively. The number of Leydig cell per testis was 15 million (Table 6).

Table 2. Morphometric data (Mean ± SEM) in gerbil.

Parameters	**Mean ± SEM**
Body weight (g)	76.87 ± 2.58
Testicular weight (g)	0.54 ± 0.03
Right testis	0.54 ± 0.03
Left testis	0.53 ± 0.03
Gonadosomatic index (%)	0.72 ± 0.04
Testis parenchyma volume density (%)	
Seminiferous tubule	92.2 ± 2.3
Tunica propria	1.5 ± 0.5
Seminiferous epithelium	85 ± 2.2
Lumen	5.6 ± 0.8
Interstitial compartiment	7.8 ± 2.3
Leydig cell	3.0 ± 0.9
Macrophages	0.3 ± 0.1
Blood vessels	2.5 ± 0.3
Lymphatic vessels	1.6 ± 1.3
Others	0,3 ± 0,2
Tubular diameter (μm)	253 ± 8
Seminiferous epithelium height (μm)	100 ± 1
Total tubular length per testis (meters)	10 ± 0.9
Tubular length per gram of testis (meters)	18 ± 1.3

Table 3. Corrected numbers of germ cell types and Sertoli cells nucleoli per cross-sections of seminiferous tubules in stage VII of SEC.

Cell types	Mean ± SEM
Type-A spermatogonia	2.4 ± 0.4
Pachytene primary spermatocytes	27 ± 2.6
Round spermatids	78 ± 8
Total of germ cells	130 ± 11
Sertoli cells nucleoli	6.3 ± 0.8

Table 4. Cell ratios obtained from the corrected counts at stage VII.

Parameters	Ratios
Pachytene spermatocytes : type A spermatogonia	12 ± 2.3
Round spermatids : type A spermatogonia	33.8 ± 5.7
Round spermatids : pachytene spermatocytes	2.8 ± 0.1
Round spermatids : Sertoli cell nucleoli	12.6 ±1.7
Total number of germ cells : Sertoli cell nucleoli	21 ± 2.8

Table 5. Sertoli cell number and daily sperm production (DSP) per testis and per gram of testis ($X10^6$).

Parameters	**Mean ± SEM**
Sertoli cell number per gram of testis	28 ± 4.3
Sertoli cell number per testis	15 ± 2
DSP per gram of testis	33 ± 5
DSP per testis	18 ± 2.7

Table 6. Leydig cell morphometry.

Parameters	**Mean ± SEM**
Nuclear diameter (μm)	8.5 ± 0.6
Leydig cell volume (μm^3)	1148 ± 266
Nuclear volume (μm^3)	328 ± 67
Cytoplasmatic volume (μm^3)	820 ± 202
Leydig cell number per gram of testis ($X10^6$)	28 ± 8.2
Leydig cell number per testis ($X10^6$)	15 ± 4

Discussion

The present study is the first to estimate the duration of the spermatogenic process and evaluate the structure of the testis through quantitative study of the gerbil. The gerbil testis weighs an average of 0.54g, constituted by approximately 92% tubular compartment and 8% interstitial compartment. Tubular compartment occupy from ~70% to ~90% of testis parenchyma in most mammals investigated (Russell *et al.*, 1990b; França and Russell, 1998). The high variability of the proportion between the two compartments is one of the factors responsible for the observed differences in sperm production in the different species (Russell *et al.* 1990a; França and Russell, 1998). Testis size is an importante parameter in the andrological evaluation of mammals. In addition to the information provided regarding normality, it also allows the inference of the rate of sperm production (Amann and Schanbacher, 1983; França and Russell, 1998; Peirce and Breed, 2001). The size of the testis is determined by a response to various factors (Young *et al.*, 1999; Young and Nelson, 2001), but the principal factor is the number of Sertoli cell, established during the period of testicular development. In humans, it was observed that about 62% of testicular parenchyma is composed of seminiferous tubules, which is less than that in the majority of species. However, the gerbil presented a higher value compared with others mammals species investigated.

A positive correlations usually exists between the tubular diameter and the spermatogenic activity of the testis (Sinha-Hikim *et al.*, 1988). In gerbil, the value observed for tubular diameter (253μm) is in the range cited for most mammals investigated (180-350μm) (Roosen-Runge, 1977). Not only does the tubular diameter vary among species but within some breeds of the same species (Okwun *et al.*, 1996). Factors such as the number of myoid cell layers in the tunica propria of the seminiferous tubule, the size and population of Sertoli and germ cells, and secretion of fluid by the Sertoli cells could determine lumen size, reflected in the final diameter of seminiferous tubule. Approximately 18 meters of tubules per gram of testis parenchyma was found in the gerbil, value related with the high tubular diameter and tubular compartment volume. In general, there are 10 - 15 meters of

tubules per gram of testis in mammals (Wing and Christensen, 1982; Neaves and Johnson, 1985; Sinha-Hikim *et al.* 1988; Queiroz, 1991; França and Russell, 1998).

The interstitial compartment of the gerbil testis is constituted by 40% Leydig cells. The organization of Leydig cells and lymph vessels in the testis varies greatly in mammals. The interstitial compartment also presents great volume variation in different species for all of the components (Griswold, 1995). There is great variation among members of the same order, family, subfamily and genus (Fawcett *et al.,* 1973). In rodents, variation are observed in capybara and rats, with the compartment occupying 50% (Paula et al., 1999) and 10% (Rocha et al., 1999) of the testicular parenchyma, respectively. Leydig cells are the main source of testosterone in adult mammals testis. The testosterone is requered for spermatogenesis, maintenance of accessory sex organ function, fertility and successful reproductive performance (Russell *et al.*, 1990a).

The organizational pattern of the seminiferous epithelium follows the typical rodent fashion (Leblond and Clermont, 1952; Clermont, 1972; Saidapur and Kamath, 1994; Muñoz *et al.* 1998). The frequency of appearance of different stages of the SEC was not identical for all stages in gerbil. The literature suggest that when the stage frequencies are grouped in premeiotic and postmeiotic phases of spermatogenesis might be phylogenetically determined among members of the same mammalian family (França and Russell, 1998; França *et al.*, 1999; Neves *et al.*, 2002). Two patterns are readily observed for rodents: species in which the premeiotic frequency is about one quarter of the entire cycle and species that present a relative equilibrium between pre and postmeiotic stage frequencies (Paula *et al.*, 1999). Gerbil shows more near the first pattern.

The duration of the cycle is considered to be a biological constant for a species, and apparentely cannot be altered by any natural factor or experimental manipulation (Clermont, 1972; Berndtson and Desjardins, 1974; Amann and Schanbacher, 1983), although strain or breed different can be found among member of same species (Russell *et al.*, 1990a). Approximately 50% species already investigated, present a cycle duration situated in an interval of 9-12 days, with about 30% below and 20% above this interval. The longest or shortest cycle duration is a factor that influency on daily sperm production (Johnson, 1986; Queiroz and Nogueira, 1992). The cycle duration found for gerbil (10.55

days) is situated in the predominant range observed for most of mammals investigated. The duration of the spermatogenuc cycle in rodents varies from 6.7 days for *bank vole* (Grocock and Clarke, 1976) to 17 days for the Chinese hamster (Oud and de Rooij, 1977). Once the duration of spermatogenesis is not necessarily the same for species closely related (Russell *et al.*, 1990a; França and Russell, 1998; Paula *et al.*, 1999), its is considered that the cycle duration is not phylogenetically determined in mammals (Neves *et al.*, 2002). In spite of the duration of spermatogenesis is controlled by germ-cell genotype (França *et al.*, 1998). However, the dogma persists that a germinal cell develops at a prescribed rate throughout spermatogenesis, and that various agents generally do not speed up or dow its progress (Clermont and Harvey, 1965; Aslam *et al.*, 1999).

During spermatogenesis, programmed cell death (apoptosis) plays an important role in limiting the germ cell population and eliminating germ cells that are defective or that carry DNA mutations, fundamental process requered for testicular homeostasia during spermatogenesis. This spontaneous germ cell degeneration result in the loss of up to 75% of the potential number of mature spermatozoa. Dysregulation of this physiological germ cell apoptosis can cause male infertility. Inappropriate germ cell apoptosis may also result from external disturbance such as alterations in hormonal support, or exposure to toxic chemicals or radiation (Oakberg, 1956; Clermont, 1972; de Rooij and Lok, 1987; Bartke, 1995; Johnson, 1995; Lee *et al.*, 1997, 1999; Sinha-Hikim and Swerdloff, 1999; de Rooij., 2001). Germ cell apoptosis during specific steps of development can be estimated comparing the ratios of germ cell numbers before and after a given development steps (Johnson, 1991). The present results found for the gerbil with only aproximately 25% formed sperm, with the highest level of cell degeneration occurs during the spermatogonial proliferative phase and cell degeneration less consequence occurs during meiotic phases of spermatogenesis process. According to the majority of mammals investigated, that spermatogenic phase in which the greatest cell loss occurred are the mitotic and meiotic phases (Roosen-Runge, 1973; Huckins, 1978; Sinha-Hikim *et al.* 1985; França and Russell, 1998; Young and Nelson, 2001; Weinbauer *et al.* 2001). The cell density of newly formed spermatocytes in different stretches of seminiferous tubules appears to be virtually the same everywhere, indicating that germ cell density is tightly controlled (de Rooij and Lok, 1987). Such

instances of degeneration could be a mechanism for the limitation of germ cells in relation to Sertoli or support cells (Johnson, 1995).

The Sertoli cell number established during the prepubertal period determines the final testicular size and the number of sperm produced in sexually mature animals (Orth *et al.* 1988; Hess *et al.* 1993). This occurs because the Sertoli cell division stops before puberty, with the Sertoli cell population becoming stable thereafter, and also because Sertoli cells can support only a limited number of germ cell, with this support capacity cell being both variable and species-specific (Berndtson and Desjardins, 1974). Since each Sertoli cell can only support a fixed number of germ cells, the Sertoli cells number is directly related to sperm count and species-specific manner (Russell and Peterson, 1984; França and Russell, 1998;). Consequently, fewer Sertoli cells will lead to smaller testes and lower number of spermatozoa produced in adult animal (Meachem *et al.*, 2001; Monsees and Schill, 2001). The ability to accommodate more germ cells may be dependent on the size of the Sertoli cell and also to a certain degree on the size and/or shape of the elongated spermatids (Russell and Peterson, 1984). Approximately 28 million Sertoli cells were found per testis gram in gerbil testis. This value is situated in the range observed in the majority of mammals between 20 and 40 million Sertoli cells per testis gram (Russell *et al.* 1990b; França and Russell, 1998). The interactions between Sertoli and germ cells are crucial to maintaining the production of normal sperm (Griswold, 1995). The Sertoli cell number is stable in the adult animal and throughout the different SEC stages, these cells are used as a reference to quantify and functionally evaluate the spermatogenic process. The apparent flexibility among species in the number of spermatids supported by a single Sertoli cell shows that, in general, species in which spermatid-Sertoli cell ratios are higher also have higher daily sperm production (DSP). The ability of Sertoli cell to support spermatids correlates positively with DSP per testis gram in these species (França and Russell, 1998). In the gerbil, this index was 12 spermatids per Sertoli cell, reaching a higher level compared to the most of rodents (Wing and Christensen, 1982; Rocha *et al.* 1999).

Daily sperm production per gram of decapsuled testis is useful in species comparisons as it is a measure of spermatogenic efficiency (Amann *et al.*, 1976; Johnson,

1986; Johnson *et al.*, 2000). The spermatogenic efficiency is usually positively correlated with the number of germ cells supported by each Sertoli cell, the volume density of seminiferous tubules, the length of spermatogenic cycle, the number of spermatogonial generations, the rate of germ cell loss during spermatogenesis, the number of Sertoli cells per gram of testis, and the size of Sertoli cells (França and Russell., 1998: Russell and Peterson, 1984). So far, the mammals spermatogenic efficiency can be grouped in: i) high spermatogenic efficiency (20 – 30 x 10^6/g), example for rabbit (26.5 x 10^6/g, Orgebin-Crist, 1968), rams (21-25 x 10^6/g, Amann and schanbacher, 1983), boars (20-24.8 x 10^6/g, Okwun *et al.*, 1996), rats (20-24 x 10^6/g, Johnson, 1986), and rhesus monkey (23 x 10^6/g, Amann *et al.*, 1976); ii) species with intermediate values, observed for stallion (17 x 10^6/g, Johnson and Neaves, 1981), bull (12.2 x 10^6/g, Cardoso and Godinho, 1985), dogs (14.5 x 10^6/g, Olar *et al.*, 1983) and cats (15.7 x 10^6/g, França and Godinho, 2003); and iii) and species with low efficiency, observed for such the human (4-6 x 10^6/g, Johnson *et al.*, 1981) and opossun (*Didelphis albiventris*) (4.8 x 10^6/g, Queiroz and Nogueira, 1992). Therefore, a mean DSP per gram of testis in gerbil (33 x 10^6/g) indicates a higher spermatogenic efficiency compared to the majority of mammals studied until the present. The lower efficiency of spermatogenesis in humans and marsupialia compared with rodents or domestic animals results from a longer duration of spermatogenesis and lower germ-cell density (Johnson, 1986; Queiroz and Nogueira, 1992). Comparisons of DSP per gram parenchyma vs. potential DSP based on germ cells in different developmental steps of spermatogenesis facilitate detection of species differences in sites of germ cell degeneration in spermatogenesis (Johnson *et al.*, 2000).

Factors such the high sperm production achieved by having relatively large testis composed of predominantly spermatogenic tissue, a short spermatogenic cycle, a high efficiency of spermatogenesis, it makes possible to the gerbil a potential model for studies of the molecular biology of the regulation of spermatogonial multiplication and stem renewal, it is useful in evaluating the effects of manipulation on spermatogenic cells, which forms the basis for studies on spermatogonial kinetics.

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CONCLUSÃO GERAL

O presente trabalho descreveu pela primeira vez a duração do CES e através de análises quantitativas avaliou a estrutura e a função do testículo do gerbilo (*Meriones unguiculatu)*. Baseado nos resultados obtidos, conclui-se:

1) a duração do ciclo do epitélio seminífero mostrou-se na média comparada com a maioria dos mamíferos;
2) devido à duração do ciclo ser relativamente rápida (10,55 dias), de o testículo apresentar alta percentagem de túbulos seminíferos, das células de Sertoli terem grande capacidade de suporte de células germinativas, verificou-se que o gerbilo é um roedor com eficiência espermatogênica alta, maior do que a grande maioria das espécies de mamíferos estudadas até o momento;
3) o gerbilo pode ser considerado modelo potencial nas investigações dos mecanismos de controle do processo espermatogênico.

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