Chapter 1 : Isolation of Sertoli Cells and Peritubular Cells from Rat Testes | Protocol

Leslie Heckert wrote: "His ideas, approaches, and results have enhanced our understanding of Sertoli cell and gamete function, improved animal models and methodologies for the study of spermatogenesis, and precipitated new concepts in testis biology, contraception and infertility.

Sex selection As early as BC, Aristotle prescribed the ligation tying off of the left testicle in men wishing to have boys. This was because people believed that the right testicle made "boy" sperm and the left made "girl" sperm. The original Latin word testis, "witness", was used in the firmly established legal principle "Testis unus, testis nullus" one witness [equals] no witness , meaning that testimony by any one person in court was to be disregarded unless corroborated by the testimony of at least another. This led to the common practice of producing two witnesses, bribed to testify the same way in cases of lawsuits with ulterior motives. Since such "witnesses" always came in pairs, the meaning was accordingly extended, often in the diminutive testiculus, testiculi. The primitive jawless fish have only a single testis, located in the midline of the body, although even this forms from the fusion of paired structures in the embryo. The right testicle is often smaller than the left. Their testes are located outside of the body, suspended by the spermatic cord within the scrotum. There are several hypotheses why most boreotherian mammals have external testes which operate best at a temperature that is slightly less than the core body temperature, e. The classic hypothesis is that cooler temperature of the testes allows for more efficient fertile spermatogenesis. In other words, there are no possible enzymes operating at normal core body temperature that are as efficient as the ones evolved, at least none appearing in our evolution so far. The early mammals had lower body temperatures and thus their testes worked efficiently within their body. However it is argued that boreotherian mammals have higher body temperatures than the other mammals and had to develop external testes to keep them cool. It is argued that those mammals with internal testes, such as the monotremes, armadillos, sloths, elephants, and rhinoceroses, have a lower core body temperatures than those mammals with external testes. It has been suggested that the ancestor of the boreoeutherian mammals was a small mammal that required very large testes perhaps rather like those of a hamster for sperm competition and thus had to place its testes outside the body. This position is made less parsimonious by the fact that the kangaroo , a non-boreoeutherian mammal, has external testicles. The ancestors of kangaroos might, separately from boreotherian mammals, have also been subject to heavy sperm competition and thus developed external testes, however, kangaroo external testes are suggestive of a possible adaptive function for external testes in large animals. One argument for the evolution of external testes is that it protects the testes from abdominal cavity pressure changes caused by jumping and galloping. Mild, transient scrotal heat stress causes DNA damage, reduced fertility and abnormal embryonic development in mice. The relative size of testes is often influenced by mating systems. In the mammalian kingdom, there is a tendency for testicular size to correspond with multiple mates e. Production of testicular output sperm and spermatic fluid is also larger in polygamous animals, possibly a spermatogenic competition for survival. Chimpanzees have high promiscuity and large testes compared to body weight 0. Human testicular size falls between these extremes 0. Amphibians and most fish do not possess seminiferous tubules. Instead, the sperm are produced in spherical structures called sperm ampullae. These are seasonal structures, releasing their contents during the breeding season, and then being reabsorbed by the body. Before the next breeding season, new sperm ampullae begin to form and ripen. The ampullae are otherwise essentially identical to the seminiferous tubules in higher vertebrates , including the same range of cell types. These are two healthy testicles. Heat causes them to descend, allowing cooling. A healthy scrotum containing normal size testes. The scrotum is in tight condition. The image also shows the texture. Testicle of a cat: Ductus deferens Testis surface The right testis, exposed by laying open the tunica vaginalis.

Chapter 2 : SERTOLI CELL BIOLOGY VOL 1 | www.nxgvision.com

Sertoli cells assist in the production of sperm in the male reproductive system. This book provides moreÂ» a state-of-the-art update on the topic of sertoli cells and male reproduction.

FTo whom correspondence should be addressed. The Sertoli cells are a strong candidate for fetal programming of future performance because the number of Sertoli cells is highly correlated with adult testicular size and the maximum rate of sperm production. Pregnant ewes were weighed weekly and lambs were weighed at birth and 2 days later. Blood was sampled at the same times. The diameter of the testicular cords did not differ. The absolute volume of testicular cords 0. Plasma follicle-stimulating hormone concentrations were not significantly affected at birth or 2 days later. We conclude that undernutrition during pregnancy can reduce testicular development in the newborn. Depending on the ability of the Sertoli cell population to recover between birth and puberty, this may limit the ultimate number of Sertoli cells and, hence, the future capacity for sperm production and fertility. Introduction effect on their future capacity to produce spermatozoa. This concept was originally developed to and the maximum rate of germ cell production Sharpe explain variations in susceptibility of humans to disease, but That is, fetal nutrition, particularly during gonadal has now been broadened and encompasses the effects of differentiation, may be a determinant of maximum capacity fetal malnutrition on pre- and postnatal development, for sperm output. Two distinct bands indicated a male fetus and a single band indicated a female fetus. Of the 38 pregnancies assessed as lar development for a review see Brown Thus, having male fetuses, 28 were selected for the study. These included 25 undernutrition during pregnancy in the sheep reduces the for experimentation and 3 replacements in case gender selection was pituitary response to exogenous GnRH in 2-month-old ram subsequently found to be incorrect. In a previous study of sheep grazing under animal house with artificial lighting imposing a photoperiod regimen similar to the natural photoperiod for this region Martin et al. From Week 10 until Week 17 of pregnancy, ewes would affect Sertoli cell numbers. We then tested whether these treatments would affect On the same day each week, before feeding, ewes were weighed testicular histology in newborn males. Each lamb was weighed within the first hour after birth, before being suckled, and Materials and methods 3 mL jugular blood was sampled. All male lambs were weighed and Experimental design bled again 2 days later. All experimental protocols conformed with the Code of Practice that Morphological examinations has been formulated by the National Health and Medical Research Council of Australia and implemented by the Animal Ethics Commit- Immediately after blood sampling at 2 days of age, lambs were given tee of The University of Western Australia. Semithin live, single fetus was confirmed and a gauge spinal needle Terumo, sections 2 mm from all blocks containing right testis parenchyma Cheltenham, Victoria, Australia was inserted into the amniotic sac. Sections were determination of fetal gender. A separate subset of 30 ewes had placed on glass slides, stained with haematoxilynâ€”eosin and examined previously undergone sampling on Day 47, but these samples had by light microscopy. Testicular g for 10 min at room temperature. The volume density Vv of testicular cords was measured USA was added to the pellet, mixed well and boiled for 10 min to lyse by point counting Weibel Briefly, a grid consisting of 64 evenly the cells and release the DNA. The number of test points overlying SCUcdO. Fifty randomly 4 weeks leading up to parturition. Testicular cords were assumed to be cylindrical and their lengths from 4. The number of Sertoli cells per testicular cord heavier in the HighME group compared with the LowME cross-section was multiplied by total testicular cord length to yield the group Table 1. In both groups, right testes were approxi- number of Sertoli cells per testis. Hormone assays Stereology Plasma concentrations of follicle-stimulating hormone FSH were Quantitative histological data are shown in Table 1. The measured in all samples, at birth 0 hours and on Day 2 after birth, absolute volume of testicular cords and the number of using a kit kindly supplied by A. Similarly, there were no significant 1. All samples were processed in duplicate in one assay for both hormones measured. Thereafter, bodyweight data were available for nine preg- nant ewes from each group. Weeks before birth Bodyweight Fig. The effect on testicular mass was not significant, Left testis weight g 0. The Discussion diameter of seminiferous tubules in adults is highly corre- lated to the number of germ cells, whereas the length of

Restricting the nutrition of pregnant ewes reduced the seminiferous tubules is highly correlated to the number of number of Sertoli cells in their newborn lambs. This Sertoli cells Hochereau-de Reviers et al. We suggest clarifies and confirms our previous work with animals that the diameter of testicular cords was not affected by studied under field conditions, where the effect on Sertoli nutrition opposite to the effect on diameter of seminiferous cell numbers was not significant Bielli et al. The tubules because gonocytes are the only type of germ cells clearer outcome of the present study is probably due to a present in immature testes and these divide slowly. However, more effective application of nutritional treatments, with a the testes of adults, but not the newborn, have large focus on the period when the reproductive axis is developing populations of proliferating germ cells and the rate of their in the fetus, a more homogeneous environment for the proliferation responds markedly to nutritional inputs. In The fact that development was specifically reduced in the addition, we cannot rule out breed differences, because Sertoli cell population also indicates the mechansims Merinos are renowned for the responsiveness of their involved. Clearly, the first candidate must be the hypo- reproductive system to nutritional inputs and may be more thalamicâ€"pituitaryâ€"testicular axis and, particularly, the responsive than the Corriedales used by Bielli et al. The capacity of newborn lambs to capacity for sperm production and fertility in the progeny. Our sampling protocol was limited to Nutrition and fetal testicular development only two samples after birth. More importantly, perhaps, we Da Silva, P. Influence of placentally mediated fetal growth restriction on the onset of puberty in male and female lambs. More serial samples will help Deligeorgis, S. Pituitary overcome the innate high degree of variation between responsiveness to GnRH in lambs undernourished during fetal life. Some of this Anim. The effects of intra-uterine et al. Whether the effects of maternal undernutrition on the Spermatogenesis and Sertoli cell numbers and function in Sertoli cells of the newborn represent another example of rams and bulls. The lambs born to underfed Blackberry, M. Morphometric and mothers clearly start out in a poor position, but the endocrine analyses of the effects of nutrition on the testis of mature Merino rams. Puberty occurring either growth correlates closely with general body growth in ram spontaneously or induced precociously in Rhesus monkey Macaca lambs Courot and Sertoli cells continue to multiply mulatta is associated with a marked proliferation of Sertoli cells. Environmental and consequences of maternal nutrition during fetal life by genetic factors affecting reproductive activity in the Merino ram. Moreover, we need to test whether undernutrition pp. Non-photoperiodic inputs into seasonal breeding in male ruminants. Determinants of the annual pattern of reproduction in mature male Uruguay. Funds for sample transportation were provided by Merino and Suffolk sheep: Blackberry was Miller, D. Suppression of supported by the National Health and Medical Research fetal gonadotrophin concentrations by maternal passive immuniza- Council of Australia. Histological slide preparation was tion to GnRH in sheep. Effects of nutrition Bielli, A. Nutritional management during fetal and post- Sharpe, R. Role of Weibel, E. Effect of maternal glucocorticoid treat- Brown, B. A review of nutritional influences on reproduc- ment on fetal fluids in sheep at 0. Manuscript received 28 May ; revised and accepted 25 July

Chapter 3 : Sertoli Cell Biology - Google Books

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Each year, this award recognizes an exemplary research career in reproductive biology. The recipient of the Carl G. Hartman Award is Michael D. Mike received a B. He conducted postdoctoral studies with Dr. He then spent two years as a Research Associate in the laboratory of Dr. Irving Fritz at the University of Toronto, where he acquired what would become a life-long interest in Sertoli cells. He has published more than original scientific articles, book chapters, and review articles. Mike is an exemplary mentor. Mike received the Frontiers in Reproduction Beacon Award in for his outstanding mentoring. One of his former students, Leslie Heckert wrote: He led by example, support and occasionally by providing unsolicited words of encouragement His lab demonstrated in that vitamin A modulated Sertoli cell function, in that vitamin A altered Sertoli cell gene expression, and in in a landmark publication with Carlos Morales, that retinol induced synchronization of seminiferous tubules in vitamin A-deficient rats. The crucial role that vitamin A plays in regulating germ cell entry into meiosis is still being investigated today not only by the Griswold lab but also by many others in the field, including Kwan Hee Kim, Peter Koopman, and David Page. These databases are freely available to the scientific community. The number of investigators across the world that have used these data bases is remarkable. As evidenced from the comments above, Mike has been at the forefront of research in male reproductive biology for the past three decades. Griswold, more is known about the role of retinoic acid in regulating meiosis and the cycle of the seminiferous epithelium, the actions of FSH and testosterone, Sertoli cell function, and the characteristics of spermatogonial stem cells. Michael Griswold meets and exceeds the criteria for the Hartman Award in terms of his original research, mentoring of many successful scientists, foresight generosity to the field, and leadership. He has made SSR proud. Submitted by Mary Hunzicker-Dunn, Ph.

Chapter 4 : Testosterone Retention Mechanism in Sertoli Cells: A Biochemical Perspective

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 Several lacunae pertaining to the mechanism of action of principal male hormone T during spermatogenesis remain to be resolved. Notably, the mechanism through which T brings about the stage-specific differentiation of germ cells lacking Androgen Receptors AR. Testosterone is a highly anabolic steroid with a rapid tissue clearance rate. Therefore, it is important to delineate the mechanism s for retention of iT, in order to understand regulation of its bioavailability in testis. Insights gained about androgen retention mechanism s from the ABPKO murine model will be of immense help in improving the efficacy of male hormonal contraceptives and infertility management. Testosterone, Androgen-binding protein, Sex hormone-binding globulin, Megalin, Sertoli cell, Spermatogenesis. The mechanism for storage of intratesticular testosterone iT , at a level several folds higher than that in circulation, however, awaits delineation. This hypothesis, however, awaits experimental substantiation. Evaluation of sperm chromatin structure by flow cytometry demonstrated that Sertoli cell AR blockade prevented initiation of chromatin condensation in elongating spermatids. AR blockade reduced the fertility of male rats due to the production of poor quality epididymal spermatozoa, deficient in thiols and protamine1 [5]. Testosterone RIAs demonstrated that tamoxifen treatment reduced the levels of intratesticular androgens in adult male rat concomitant with a reduction in their siring ability [6]. Testosterone can also act to regulate spermatogenesis via its non-aromatizable metabolite, Dihydrotestosterone DHT and estradiol E2 , its aromatizable metabolite [7 , 8]. Plasmatic hormone RIAs also demonstrated that a circadian E2 acrophase occurred in human subjects between h [4]. The role of E2 in fertility regulation became evident from several studies. Estrogen Receptor ESR1 gene null mutation led to sterility in mice [9]. Steroid hormone RIAs demonstrated that high intratesticular E2 iE levels produced in E2-treated rats reduced intratesticular androgens, disrupted the formation of Tubulobulbar Complexes TBCs and led to spermiation failure. This study revealed plasmatic E2 uptake by the Sertoli cells [10 , 11]. Histology of testes of estradiol-treated rats revealed a reduction in the height of Sertoli cells, attributed to lack of polymerization of cytoskeletal protein Vimentin [12 , 13]. Confocal Microscopy subsequently confirmed disorganization of Sertoli cell Vimentin in E2-treated rat testis [14]. ESR1 agonist reduced the sperm counts, evaluated by flow cytometry of testicular cells, through suppression of plasmatic gonadotropins and testosterone. Reduced T levels led to arrest of conversion of round to elongating spermatids, owing to downregulation of chromatin condensation proteins. It is tempting to suggest that E2 could be playing an autoregulatory physiological role in the predominantly androgen-dependent biological process of spermatogenesis. Significance of High Concentration of Intratesticular Testosterone IT Intratesticular T is the most decisive hormone for maintenance of qualitative spermatogenesis in mammals [17]. Natural and genetically engineered mutant mice have contributed to the delineation of T- dependent stages of spermatogenesis. Congenital deficiency of T in hpg mice blocked the first meiotic division and arrested spermatogenesis at pre-meiotic spermatocyte stage, reversible with T implants [18]. The significant finding that emerged from T supplementation studies was that Sertoli cells internalized steroidal molecules from the peripheral circulation. Genetic mutant studies suggested the involvement of Sertoli cell AR in mediating T effect on round spermatid adhesion and development. The arrest of spermatogenesis at pachytene stage in Androgen Receptor Knockout ArKO mice indicated the role of AR in adhesion of round spermatids to Sertoli cells [19]. Androgen Response Elements ARE were demonstrated by chromatin immunoprecipitation in the promoters of Sertoli cell genes, namely phosphatidylinositol binding clathrin assembly protein, early endosomal autoantigen1 and syntaxin, in the testis of estrogen-treated rats [23]. Thus, high levels of iT are essential for mediating its molecular effects via

Sertoli cell AR. Significance of Intratesticular Testosterone IT Storage Mechanism to Spermatogenesis Several studies suggested that a functional relationship exists between iT levels in Sertoli cells and differentiation of spermatozoa. CHIP chromatin immunoprecipitation assay demonstrated that T and its metabolites regulated testicular genes involved in actin remodeling and endocytosis, in the testis of E2 treated rats. The presence of a storage protein could be crucial for ensuring T bioavailability for gene transcription during spermatogenesis. Histological and Confocal Microscopic evaluation of the testis of E2-treated rats revealed that reduction in iT levels had affected organization of Sertoli cell cytoskeletal Vimentin [12 - 14]. Flow cytometric evaluation of monobromobimane mBBr fluorescent dye uptake by epididymal sperm, taken from CPA- and E2-treated rats, indicated a reduction in sperm thiols, thus an altered oxidation status [5 , 26]. The occurrence of plasmatic T internalization emerged from studies of T 0. T 24cm implants restored the arrested cytodifferentiation within four days, ostensibly by internalizing T from peripheral circulation [7]. Failure of this restorative effect to occur in the presence of either flutamide AR Antagonist or L, 5alpha-reductase inhibitor revealed the significance of bioavailability of intratesticular DHT iD [8]. Several studies indicated the existence of an FSH-dependent mechanism that modulates androgen responsiveness of Sertoli cells [27 - 29]. Immunohistochemical localization of Bromodeoxyuridine BrdU in spermatogonial DNA of E2-treated rat testis had demonstrated its mitotic role [12]. FSH apparently produced a mitotic effect on spermatogonia via aromatization of iT to iE. Real-time PCR studies in rats treated with specific agonists of estrogen receptors demonstrated a direct testicular role of iE. Therefore, maintenance of high iT levels is necessary for the synthesis of iD and iE required for efficient spermatogenesis. A mechanism for storage of T in the testis would be of physiological relevance due to its lipophilic nature, high tissue clearance rates and circadian secretion. Mechanistic Role of Androgen-Binding Protein s in Testosterone Retention Sub-human mammals express a specific androgen-binding protein ABP of hepatic and testicular origin, besides a non-specific albumin carrier protein for plasmatic T [2]. Albumin and androgen-binding proteins present in Systemic Circulation sequester T. ABP and SHBG are high-affinity, androgen-binding proteins, expressed from a conserved shbg gene, in a tissue-specific manner, in human and sub-human mammals, respectively. Sertoli cells secrete ABP bidirectionally into serum and seminiferous tubular fluid in rats, regulated by FSH [3 , 34 , 35]. Since human SHBG is of hepatic origin, the underlying reason for CREM-induced expression of a steroid-binding shbg transcript, in the acrosomes of human spermatids, is not comprehensible [36]. Radioimmunoassays detected ten-fold higher iT as compared to plasmatic T. This feature, common to all mammals, is suggestive of the existence of a common physiological mechanism for iT retention and storage in Sertoli cells. SHBG is purported to mediate plasmatic T signals via alternative routes [37]. Megalin is a transmembrane receptor involved in uptake of sex steroids in tissues. Megalin deficiency was immunohistochemically confirmed in the testis of megalin null mice. Megalin null mice present with cryptorchidism. Male Megalin null mutant mice have reduced expression of several androgen inducible genes namely, Tex12, Morc, Stk25, Ramp2 and increased expression of androgen-repressed genes namely, Mpo, Igfbp5 [38]. SHBG can bind and transport plasmatic T into sex-steroid dependent tissues via Megalin receptors [39]. Transgenic mice overexpressing ABP in Sertoli cells, expressed the protein from 5. Histological assessment of ABP h transgenic mouse testis revealed apoptosis of germ cells arrested at meiotic stage. These pathophysiological effects are characteristic of E2 exposure, seen in rats treated with specific ESR1 and ESR2 receptor agonists [15 , 33 , 44 , 45]. ABP transcripts were downregulated in CPA-treated rat testis, ostensibly by accelerating autophagic clearance [5 , 46]. ABP transcripts were also upregulated in E2-treated rat testis albeit downregulated in tamoxifen- estrogen receptor antagonist treated rat testis [26 , 47]. These studies suggest an autoregulatory role of iT and iE in iT retention and regulation of bioavailability for spermatogenesis. The observed downregulation of the transcripts of several testicular genes, involved in the process of spermatid chromatin condensation during spermiogenesis, in CPA-treated rats, supports this logic [24]. Indeed, the observed upregulation of ESR1 and aromatase, concomitant with meiotic arrest and germ cell apoptosis in testis of ABP transgenic mice were pathophysiological estrogenic effects of ABP overexpression [44 , 45 , 48]. Ostensibly, gene overexpression approach failed to demonstrate the physiological role of ABP in iT retention and storage. Development of mice lacking androgen-binding protein would be the ideal

approach to study its role in iT retention and storage [3]. However, in order to overcome the potential developmental problems of viability and cryptorchidism, a conditional knockout of Sertoli cell shbg gene would be an appropriate approach to study the role of ABP in iT retention and spermatogenesis. The 3Kb coding region expresses a 1. Cleavage of a signal peptide from the N-terminus of the precursor protein generates a mature 4. Photoaffinity labeling identified the steroid-binding region in residues in rat protein [50]. Expression of human SHBG deletion mutants in E coli identified amino acid residues to be involved in steroid binding [2 , 51 , 52]. Two promoters regulate tissue-specific expression of murine ABP [53 , 54]. P1 promoter expresses the protein from exons in the testis. An alternative promoter upstream of P1 expresses hepatic and cerebral ABP [35 , 55 , 56]. The androgen-binding protein essentially exists as a dimer comprising differentially glycosylated protomers [57]. Signal peptide cleavage in the endoplasmic reticulum during translation produces a mature protein comprising amino acids [35 , 49]. The mature protein undergoes post-translational modifications and is N-glycosylated at two positions. Glycosylation ensures a secretory role but is not a pre-requisite for steroid binding [57 , 60]. The steroid-binding site of SHBG in each protomer is highly conserved in amino-terminal LG domain encoded by exons Therefore, development of mice lacking the steroid-binding domain would be an effective strategy for studying the role of ABP in T retention, iT storage and spermatogenesis. Most importantly, retained glycosylation sites will not affect the secretion of the mutant protein. Southern blotting, immunohistochemistry, Western blotting and RIA will confirm the efficacy of ablation of shbg transcripts and ABP protein. The mutant model will also provide information about the independent role, if any, of ABP molecule in spermatogenesis. A systematic evaluation of iT targets in conditional ABPKO mice lacking T binding domain will reveal the effects of breakdown of the mechanism of iT retention and storage on spermatogenesis at adulthood. Leydig cells secrete lipophilic hormone T in peripheral circulation [64 , 65]. T ostensibly could interact with Sertoli cells through several pathways. Free T could enter Sertoli cells by diffusion but its lipophilic nature would limit intracellular bioavailability [61]. A more plausible pathway for T to enter Sertoli cells would be via endocytosis of vascular androgen-binding protein s. The binding protein s would solubilize the lipophilic T and that would ensure bioavailability at nuclear AR. Indeed, sex steroid dependent tissues express transmembrane receptor megalin for endocytosis of plasmatic sex steroids. Sertoli cells do internalize sex steroids via megalin since Megalin receptor null mice have developmental defects like cryptorchid testis [38]. Sertoli cell aromatase and 5alpha-reductase enzymes aromatize or reduce iT to iE and iD, respectively. Germ cells express ER but not AR. ABP would transport iT to germ cells for aromatization to generate E2. It could also protect germ cells from excess iE exposure akin to protection of developing fetus from overexposure to T. Schematic mechanism for androgen retention in rat Sertoli cells.

Chapter 5 : Testicle - Wikipedia

Sertoli cells extend from the basal lamina to the lumen of tubules, and adjacent Sertoli cells envelop and provide a structural scaffold for germ cells as they differentiate within the tubule (Fig.). 9 Undifferentiated spermatogenic stem cells, called spermatogonia, lie along the basal lamina at the periphery of tubules, interspersed between Sertoli cells.