

Postulated Human Sperm Count Decline May Involve Historic Elimination of Juvenile Iodine Deficiency: A New Hypothesis with Experimental Evidence in the Rat

James W. Crissman,^{*1} Paul S. Cooke,[†] Rex A. Hess,[†] M. Sue Marty,^{*} and Ashley B. Liberacki^{*}

^{*}Health and Environmental Research Laboratory, Building 1803, The Dow Chemical Company, Midland, Michigan 48674; and [†]University of Illinois, Department of Veterinary Biosciences, Urbana, Illinois 61802

Received March 4, 1999; accepted June 28, 1999

Human sperm count studies, historic dietary iodination, and an animal model where neonatal goitrogen administration causes unprecedented testis enlargement, together suggest an hypothesis relevant to the postulated fall in human sperm counts. We present the hypothesis with a supporting study extending the model to include iodine deficiency. In a one-generation rat reproduction study, dams were fed an iodine sufficient (control, 200 ppb I) or deficient (low iodine diet [LID], <20 ppb I) diet from prebreeding through weaning, when male offspring were divided into three groups: 1) controls from iodine sufficient dams, 2) neonatal LID (NLID) from the LID dams, fed control diet postweaning, and 3) chronic LID (CLID) from LID dams, fed a moderate LID (40 ppb I) postweaning. F1 males were euthanized on postnatal day (PND) 133 ± 1. Each of the three diet groups comprised two subgroups in which testicular parameters were evaluated: 1) daily sperm production (DSP), sperm motility, morphology, and histopathology, and 2) Sertoli cell and round spermatid morphometry. In the first subgroup, NLID and CLID testes weights were 8.5% and 14.0% heavier than their unusually heavy controls (3.921 g; historical control mean ≈ 3.5 g), with proportional DSP increases. Sperm motility, morphology, and testis histopathology were unaffected. In the morphometry subgroup, respective increases in NLID and CLID rats included testes weights (+28.6% and +20.3%), Sertoli cells (+24.8% and +23.9%), and round spermatids (+20.4% and +15.8%). The results indicate that neonatal iodine deficiency can significantly increase spermatogenic function in rats, and support our hypothesis concerning human sperm counts.

Key Words: iodine deficiency; hypothyroidism; sperm count; sertoli cells; rat; human; testes.

The Sperm Count Debate

Based on meta-analysis, it has been postulated that sperm counts have gradually fallen 40–50% over the last 50 years (Carlsen *et al.*, 1992). Swan *et al.* (1997) selected 56 of the 61 sperm count studies used in Carlsen's study, separated them by

country, reanalyzed them, and also concluded that sperm counts in humans have fallen. However, Olsen *et al.* (1995) explored the shape of the curve defined by Carlsen's collected studies and suggested that any change in sperm counts was more sudden. Recently, Bonde *et al.* (1998) demonstrated an association between year of birth and falling sperm counts in a meta-analysis of 10 Danish occupational studies. However, other geographically based data cast doubt on any global decline (Fisch *et al.*, 1996; Fisch and Goluboff, 1996; Paulsen *et al.*, 1996; Saidi *et al.*, 1999).

In their meta-analysis of 61 collected publications, which appeared from 1938 to 1990, Carlsen *et al.* (1992) calculated a straight downward sloping line, which if extended forward or backward creates opposite but equally alarming scenarios. However, additional analysis (Olsen *et al.*, 1995) showed that the line with the least residual error was not straight but was a stair-step or "waterfall" model, with a flat line through about the first 25 years, then a precipitous drop, then a second flat line through the last 25 years to the present. Their speculation about the apparent drop concerned social changes, reporting bias, differences in methods over time, and the possibility of a sudden worldwide environmental change. Concerning the most recent 25 years, there are conflicting data to fuel the debate, and no consensus has been reached as to whether sperm counts are currently going up, down, or not changing (Auger *et al.*, 1995; Bonde *et al.*, 1998; Brake and Krause, 1992; Fisch *et al.*, 1996; Fisch and Goluboff, 1996; Paulsen *et al.*, 1996; Saidi *et al.*, 1999; Sherins, 1995; Wittmaack and Shapiro, 1992). Besides the geographical variation noted above, it is not possible to correct the previously collected data for all of the important, but uncontrolled or poorly controlled variables that have been acknowledged in the literature, including season (Tjoa *et al.*, 1982), period of abstinence, age, selection bias, and counting methods (Olsen *et al.*, 1995; Swan *et al.*, 1997). Other uncontrolled variables include population changes in racial composition, various clothing and lifestyle effectors of scrotal temperature, and a marked increase in obesity. We propose adding juvenile iodine and thyroid hormone status to this list.

¹ To whom correspondence should be addressed. Fax: (517) 638-9863. E-mail: jcrissman@dow.com.

The Thyroid Effect on Testis Development

An animal model has been developed in which there is a dramatic increase in testicular size and spermatogenic function. By giving the potent goitrogen 6-propyl-2-thiouracil (PTU; 0.1%) in the water of the dam (and from the dam to the pup via the milk) from birth to weaning on postnatal day (PND) 25, the neonatal rat rapidly becomes hypothyroid (critical period PND 4–16), evidenced by markedly depressed serum T4 levels and T3 levels that are decreased about one-third (Cooke and Meisami, 1991). In neonatal testes, Sertoli cells are rich in thyroid hormone receptors (Jannini *et al.*, 1990), and thus there are important effects of hypothyroidism in this tissue. The deficiency of thyroid hormone retards Sertoli cell maturation. This is the key to understanding the ultimate effect on the testes: the delay prolongs the period of proliferation, and thus ultimately *increases* the number of Sertoli cells (van Haaster *et al.*, 1992).

After PTU is stopped (PND 25) and the euthyroid state is recovered (PND 45–50), the maturing animals subsequently develop testes that, in the optimized model, become almost twice normal size and produce more than twice the number of normal and fertile sperm (Cooke and Meisami, 1991). Specifically, a 157% increase in the number of Sertoli cells was observed in 90-day-old Sprague-Dawley rats (Hess *et al.*, 1993). The number of Sertoli cells determines the number of spermatogenic clonal units that can be supported, and thus the size and sperm-producing capacity of the testicle. On a per gram of testis basis, spermatogenic efficiency was increased 25% or more (Cooke *et al.*, 1993). The mean diameter and length of the seminiferous tubules were increased, creating a 60% increase in mean tubular volume (Hess *et al.*, 1993). Testis weight, which normally plateaus at 90–100 days, did not reach its maximum until after PND 135, with an 80%+ testis weight increase over controls (Cooke and Meisami, 1991). At the end of the treatment period (weaning) rat pups weighed about half as much as controls. During the subsequent growth period, weight gains paralleled controls, but body weights remained about 20% less than controls at maturity. Like Sertoli cells, the number of Leydig cells also increased, but the cells were smaller, and serum testosterone levels were unaffected (Cooke, 1991).

The effect of neonatal hypothyroidism on the testes is not an artifact of PTU treatment nor peculiar to the rat. Similar effects have been observed in mice (Joyce *et al.*, 1993), hamsters (Kirby *et al.*, 1993), and chickens (Kirby and Mankar, 1995). Other chemical goitrogens, methimazole (Cooke *et al.*, 1993) and polychlorinated biphenyls (PCBs) (Cooke *et al.*, 1995), cause the same effect. Replacing thyroxine while treating with a goitrogen eliminates the testes' response (Cooke *et al.*, 1995; Cooke and Meisami, 1991).

Connecting the Animal Model to Historic Iodination

Based on the animal model, we hypothesized that dietary iodine deficiency and its accompanying hypothyroidism may

have led to higher sperm counts in men born prior to the widespread introduction of iodized salt. This led to the question of whether the sperm count meta-analysis put forward by Carlsen *et al.* (1992) as reanalyzed by Olsen *et al.* (1995) could be temporally correlated with the epidemiology and history of iodine deficiency. The average man in the Carlsen study was 31 years old. Men born in 1924, the year iodized salt became available in the United States, turned 31 in 1955. There was up to a 10-year delay from the time of data collection until publication in the papers cited by Carlsen. Thus, if the neonatal hypothyroid model holds true for man, one might predict a drop in sperm counts during the baby-boom years of the late 1950s and well into 1960s, as the tested population included fewer and fewer men who had been iodine deficient in their early lives. Olsen's stair-step model estimated the year of the postulated fall at 1964.

We therefore hypothesize that if sperm counts have indeed fallen in men in the developed world, the phenomenon may have been at least partially due to optimization of human iodine and thyroid status. Iodination of salt in the mid-1920s led, decades later, to the attrition of reproductive-age men who had experienced a degree of childhood hypothyroidism—a condition that may have caused larger testes and higher sperm counts in a large portion of them. The present study extends the animal model for this proposed effect.

MATERIALS AND METHODS

Test species and husbandry. Male and female CD rats were purchased from Charles River Breeding Laboratory (Kingston, NY). Females were obtained at 4 weeks of age; the males, obtained at 10 weeks of age, were used for mating purposes only and received the test diets only during cohabitation with the females. Upon arrival at the laboratory (fully accredited by AAALAC International), all rats were examined for health status by a veterinarian. Male rats were allowed to acclimate to the laboratory environment (12 h light, 12 h dark, 40–70% humidity, $22 \pm 1^\circ\text{C}$, air flow 12–15 changes/h) for approximately 2 weeks prior to mating. Females were placed on test diets on the day of arrival.

For the randomization procedure, the rats were weighed and ranked according to body weight, and those from the extremes of the distribution were removed from the population until only the number of animals required for the study remained. These rats were randomly assigned by weight to the treatment groups. Rats were identified by subcutaneously implanted coded transponders (Biomedic Data Systems, Maywood, NJ).

During the acclimation, pre-mating, early gestation (days 1–18), and post-weaning period, rats were housed singly in wire mesh, stainless steel cages. During the mating period, rats were housed one male and one female per cage as described above. From approximately day 19 of gestation, and throughout the lactation phase of the study, females and litters were housed in plastic cages provided with ground corncob nesting material.²

Females were fed *ad libitum* either a low iodine diet (LID, <20 ppb I) or the control diet, which was the same LID supplemented with potassium iodate (KIO₃) to provide at least 200 ppb I, and distilled water throughout the study. Iodine content in the diets was determined by epithermal neutron activation analysis at several points prior to and during the study. Breeding males were

² Corn-cob bedding material analysis for iodine detected <15 ppb I, the limit of detection in this material.

given Certified Lab Diet #5002 (PMI Feeds, St. Louis, MO) and municipal tap water *ad libitum* except during the mating period, at which time they received the same feed and water given to the females.

Low iodine diets. The LID was a cornstarch-based, L-amino acid–defined, powdered, rodent diet (Dyets Inc., Bethlehem, PA, #515757) without added iodine. Iodine in the LID was below the level of detection (10 ppb). The control diet was formulated as follows: potassium iodate (0.136 g KIO₃, Sigma Chemical, lot 16H1084) and 800 g sucrose (Sigma Chemical, lot 115H07321) were mixed to make a 0.1 mg I/g premix. Two grams of this premix were added to each kilogram of the Dyets, Inc. LID (#515757) to give a 200-ppb diet. Four samples of this diet were analyzed, and iodine was detected at 54 ± 8 ppb to 120 ± 10 ppb.

Due to unexpected postweaning pup losses, an additional diet, fed to the chronic LID subgroup of F1 males after weaning, was formulated using the same premix to add 40 ppb I. Epithelial neutron activation analysis of two samples of this diet gave values of not detectable at 10 ppb and 35 ± 8 ppb iodine.

Breeding procedure. Each female was placed with a single randomly selected male until pregnancy occurred or until 2 weeks had elapsed. Dams were observed once daily during cohabitation for positive signs of copulation. Daily vaginal lavage samples were evaluated for the presence of sperm as an indication of mating. The day on which sperm were detected or a vaginal copulatory plug was observed *in situ* was considered day 0 of gestation. The sperm- or plug-positive (presumed pregnant) females were then separated and returned to their individual cages. On day 19 of gestation, each pregnant female was placed in a nesting cage. The adult females continued to receive their respective diets throughout gestation and lactation and until all litters on study were weaned. After the mating periods, females who failed to mate and all of the males were humanely euthanized by CO₂ inhalation and discarded.

Pup and litter culling. All 14 LID litters, and all nine control litters with two or more males were used. Female pups were used to obtain a uniform litter size of five following the day 4 sacrifice and culling. Within the LID group, two male neonates were fostered into each of two litters to obtain a usable number of pups. Those LID litters with only two males contributed one to the NLID and one to the CLID sperm parameters subgroup. LID litters with three or four males were used similarly, with an additional male necropsied at weaning (PND 21). LID litters with five or more males contributed two each to the NLID and CLID groups to populate both the sperm parameter and testes morphometry subgroups, as well as one male that was necropsied at weaning. All nine control litters had three or more males and so contributed one male for necropsy at weaning, one for the sperm parameters subgroup, and up to one for the morphometry subgroup. All other litters (dams and pups) not selected were culled from the study.

Study design. (Fig. 1) The dams, comprising 20 control and 30 LID female rats, were maintained on their respective diets for the entire study. The LID group was larger to compensate for possible reductions in fertility.

After 90 days on the diets, when serum T4 levels in the LID dams had reached undetectable levels, they were mated and delivered their litters. The F1 pups were weaned on PND 21. At weaning, one randomly selected F1 pup from each of the 9 control and 13 LID litters (one of the 14 LID litters had only enough males for the terminal sacrifice) was submitted for necropsy and serum thyroid hormone determinations. Additionally, two randomly selected F1 male pups/litter from the control group and four F1 male pups/litter (if available) from the LID group were selected for continuation on test. The four F1 male pups/litter from the LID group were randomly assigned to two diet groups (two pups/litter/group), neonatal LID (NLID) or chronic LID (CLID). The F1 males assigned to the NLID group were fed the iodine-supplemented control diet from weaning until study's end. The F1 males assigned to the CLID group continued to receive a low iodine diet; however it was necessary to supplement the LID with a small amount (40 ppb) of iodine because of postweaning pup mortality. As F1 male rats reached 133 ± 1 days of age, a complete necropsy was performed on one F1 male from each litter represented in each diet group (control, NLID, CLID); these rats comprised the sperm parameters subgroup. Simultaneously, the testis morphometry subgroup, consisting of an additional

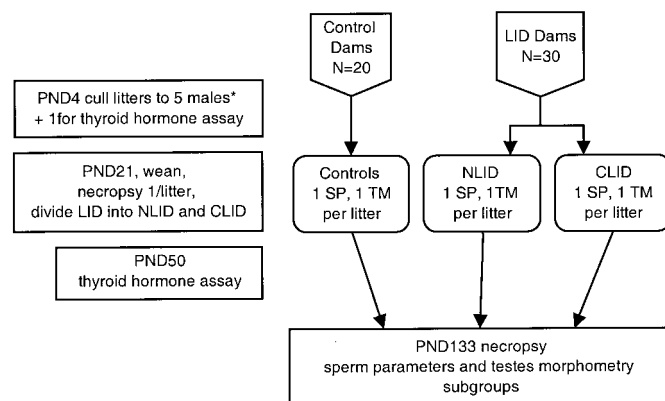


FIG. 1. LID litters divided at weaning into NLID and CLID groups. CLID, chronic low iodine diet (remained on LID until study end); LID, low iodine diet; NLID, neonatal low iodine diet (given iodine supplemented control diet after weaning); PND, postnatal day; SP, sperm parameters; TM, testis morphometry. Each group includes sperm parameters and testes morphometry subgroups. Major data collection points at PND 4, 21, 50, and 133. Actual age at PND 50 and PND 133 was ± 1 day. *Litters culled to five males if available, or litter size equalized with females.

five F1 males from each diet group (one each from five randomly preselected litters), was submitted for perfusion fixation and necropsy.

Physical observations. Throughout the test period, each rat on study was observed twice daily for clinical abnormalities. All pups found dead, or pups that were euthanized in a moribund condition, were examined grossly for possible defects and/or cause of death and discarded.

Body weights and feed consumption. All adult females had body weights and feed consumption recorded weekly during the prebreeding period. Sperm-positive females were weighed on days 0, 7, 14, and 21 of gestation. Females that delivered litters were weighed on days 1, 4, 7, 14, and 21 of lactation. During breeding, feed consumption was not measured because of cohabitation with males. During gestation, feed consumption was measured weekly in sperm-positive females. After parturition, feed consumption was measured on days 1, 4, 7, 11, 14, 17, 19, and 21 of lactation.

Litter data. Litters born overnight were considered to have been delivered on the morning they were found, and were designated as PND 0. All litters were examined as soon as possible after birth. For each litter, the number of live and dead pups on PND 0, 1, 4, 7, 14, and 21; the sex and weight of each pup on day 1; and the weight on days 4 (before and after culling), 7, 14, and 21 of lactation were recorded. Any visible physical abnormalities or demeanor changes in the neonates were recorded at the time they were observed.

Necropsy. Following weaning of pups, dams were given a complete gross necropsy. Under light methoxyflurane anesthesia, blood was collected from the retro-orbital sinus for serum T4 determination. The dams were then weighed, deeply anesthetized, and euthanized by decapitation. Weights of thyroid, brain, and kidneys were recorded. Thyroid glands were dissected and weighed following fixation in neutral, phosphate-buffered, 10% formaldehyde, and processed for routine histopathology. The remaining tissues were not saved. Male F1 pups designated for PND 21 necropsy were treated similarly, except that organ weights included testes.

On PND 133 ± 1, necropsy was performed on F1 males in the sperm parameters and morphometry subgroups. For the sperm parameters subgroup, blood was collected under light methoxyflurane anesthesia for serum T4; the rats were weighed, deeply anesthetized, and euthanized by decapitation. Weights for the thyroid gland, kidneys, brain, testes, and epididymides were recorded. From the right epididymis, a sperm sample was evaluated for motility at the time of necropsy, and smears were made for later evaluation of sperm morphology. The left testis was frozen for later determination of DSP.

The remaining tissues were saved in neutral buffered 10% formalin. The right testis was examined by routine histologic techniques.

Rats in the morphometry subgroup were fasted overnight, weighed, heparinized, anesthetized with methoxyflurane, and perfused *in situ* with 0.7% sodium nitrate in 0.05 M phosphate buffer, followed by a phosphate-buffered solution of 1.5% glutaraldehyde–4% formaldehyde (c. 540 mOs). Tissues were stored in the same fixative. Tissues were examined for gross abnormalities. Perfused weights for the thyroid gland, kidneys, brain, testes, and epididymides were recorded. Both testes were examined morphometrically.

Serum thyroid hormones. Blood was obtained for serum T4 and T3 determinations from one male/litter/group, during the in-life period (PND 50 ± 1) and at the three termination times (PND 4, 21, and 133 ± 1). Blood samples were obtained on PND 4, from one randomly chosen male/litter from litters having at least three (controls) or five (LID) male pups, by anesthetizing the pups with methoxyflurane, opening the thoracic cavity, and nicking the left atrium of the heart. Blood was then captured with serum separator tubes (Microtainer, Becton Dickinson, Franklin Lakes, NJ). Blood from PND 21 and older rats was obtained as noted above. Serum T4 was determined at the Dow laboratory using the Boehringer Mannheim T4 System Pack enzyme immunoassay run on the Hatachi 914 automatic analyzer. This system has a reportable range of 0.5–20.0 µg T4/dl.

Serum thyroid hormones were determined at the University of Illinois laboratory using commercially available radioimmunoassay kits (Coat-A-Count Total T4 and Coat-A-Count Total T3, Diagnostic Products Corp., Los Angeles, CA), which utilize antibody-coated tubes and 125I-labelled thyroxine and triiodo-L-thyronine, respectively. The T4 assay was run following manufacturer's instructions except sample size was increased to 50 µl and incubation time increased to 1.5 h. Assay sensitivity was 0.07 µg T4/dl, with intra-assay and interassay coefficients of variation of 3.0% and 3.5%, respectively. The T3 assay was run following manufacturer's instructions. Assay sensitivity was 0.007 µg T3/dl, with intra-assay and interassay coefficients of variation of 3.0% and 9.0%, respectively.

Testis morphometry. Histomorphometric analysis of the testis was conducted by a slightly modified optical dissector method (Wreford, 1995). Each testis was cut into nine evenly spaced transverse slices, then three slices to be embedded were chosen by randomly picking one of the first three, and then every third slice—e.g., if slice 2 (random), then slices 5 and 8. Thick sections were cut at a nominal thickness of 20 µm, and stained with PAS/hematoxylin. An approximate hemisection, avoiding section artifacts, was manually outlined on each testis slide with the computerized optical dissector system, which then picked 100 evenly spaced sampling sites.

Sertoli cell and round spermatid numbers were determined using optical dissector stereology (Meachem *et al.*, 1996). This method has a low bias because there is no assumption that nuclei are spherical, therefore it is valid for counting Sertoli cells that exhibit pubertal changes in shape and size. Each thick section was examined by focusing through the central approximately 10-µm thick plane and counting nuclei within this zone as their equatorial regions appeared in focus (Meachem *et al.*, 1996). Numerical density for each cell type was calculated by number of cells counted per volume of the dissector area. Testis density was assumed to be 1.0; therefore, the number of cells per pair of testes was simply calculated from the number of cells in the counted volume and the testes weight.

Daily sperm production. Testes were decapsulated, weighed, and homogenized (Cooke *et al.*, 1991; Robb *et al.*, 1978). Step 17–19 spermatids survive this homogenization and can be counted in a hemocytometer. In the rat, developing spermatids spend about 6.3 days in these steps (Clermont and Harvey, 1965). Thus, DSP was calculated by dividing the number of spermatids determined, on a per-testis basis, by 6.3.

Sperm motility. Sperm motility was assessed by the method of Toth (1992). The right epididymis along with part of the vas deferens was clamped with a hemostat at approximately the corpus cauda junction. The cauda was then gently nicked with a scalpel blade to allow sperm to emerge from the engorged cauda epididymis, which was then dipped into a plastic Petri dish (35 mm) containing prewarmed incubation medium (Medium 199, containing

Hank's salts, L-glutamine [Life Technologies, Catalog number 11151-040], and 0.5% bovine serum albumin). Sperm released into the medium were incubated for approximately 3 min with intermittent gentle swirling to disperse them throughout the suspension. Following the 3-min incubation, an aliquot of the sperm suspension was taken using 100 µl capillary tubes and loaded into the analyzer. A minimum of four fields containing sperm was evaluated, resulting in a count of at least 200 sperm, and analyzed for motility using a Hamilton-Thorne IVOS Version 10 Semen Analyzer. Motility was reported as an average percent motile and average percent progressively motile, as defined by Seed *et al.* (1996).

Sperm morphology. Methods to assess sperm morphology followed the principles of Filler (1993). One drop of the suspension of live sperm from the motility assessment was placed on a glass slide, and a smear was made using the wedge method commonly used for preparing blood smears. The slides were air dried and stained by immersion in eosin Y at room temperature, rinsed to remove excess stain, and again air dried. Approximately 200 sperm from each rat were examined by light microscopy and classified as normal or abnormal.

Statistical evaluation. Body and organ weights, DSP, and testis morphometry data were first evaluated by Bartlett's test for equality of variances; based upon the outcome, either a parametric or nonparametric analysis of variance (ANOVA) was done. If the ANOVA was significant, a Dunnett's test or the Wilcoxon Rank-Sum test with Bonferroni's correction was performed. Gestation length, average time to mating, litter size, and sperm motility, were analyzed using a nonparametric ANOVA; if significant, the Wilcoxon Rank-Sum test with Bonferroni's correction was done. Outliers were excluded from analysis only for documented, scientifically sound reasons. The fertility indices were analyzed by the Fisher exact probability test, with Bonferroni's correction where appropriate. Evaluation of the neonatal sex ratio was by the binomial distribution test. Survival indices and other neonate incidence data were analyzed, using the litter as the experimental unit, by the modified Wilcoxon test.

RESULTS

In-Life Observations

There were no spontaneous deaths nor observed morbidity among the parental generation rats during the study. Five juvenile F1 male rats from four different litters in the CLID group died approximately 1 week after weaning. Typically they stopped eating and had markedly decreased motor activity for a day or less before dying. Subsequently, 40 ppb I was added to the diet of the CLID group, and no further spontaneous death occurred. We considered this added measure of iodine, with the small amount naturally present, to comprise a moderately iodine deficient diet, a judgment later supported by the increases in terminal thyroid gland weights that were much smaller than in the LID dams (Janssen *et al.*, 1994).

Reproductive Indices and Pup Survival

The fertility index (the percent of mated females that delivered a litter) was low but not statistically different: 45.0% for controls and 56.7% for the LID dams (Table 1). Gestation survival index (percent born alive) and PND 4 preweaning survival was similarly high for both groups. No nursing pups died after the PND 4 culling.

The LID litters were significantly smaller (8.0 ± 3.4 pups) than control litters at birth (12.3 ± 4.3 pups). The LID litter size was also below our laboratory historical control range,

TABLE 1
Reproductive Indices in Control and Iodine Deficient (LID) Dams

Diet group (number of dams)	Control (20)	LID (30)
Female fertility index ^a	45.0%	56.7%
Copulation index ^b	80.0%	76.7%
Gestation survival index	100.0%	97.1%
	(111/111)	(136/140)
Litter size (mean \pm SD)	12.3 \pm 4.3	8.0 \pm 3.4 ^c
PND 4 preculling survival index	92.8%	93.4%
Sex ratio day 1 (male:female)	46:54	56:44
Gestation length (days)	22.0 \pm 0.0	22.2 \pm 0.7
Time to mating (days)	2.9 \pm 2.6	3.9 \pm 3.0

Note. LID, low iodine diet; PND, postnatal day.

^a No. of females that delivered litter/no. females cohoused with male \times 100.

^b No. females mated (sperm positive, vaginal plug, or delivered a litter)/no. females cohoused with male \times 100.

^c LID dams delivered smaller litters ($p < 0.05$). There was no additional mortality of nursing pups following the PND 4 culling.

suggesting that this was an effect of iodine deficiency. There were no control pups born dead; two pups out of the 17 litters in the LID group were stillborn. Fourteen of the LID litters had an adequate number of male pups for the study. With similar postpartum pup losses, the significant difference in litter size was maintained until the PND 4 culling.

Feed Consumption (data not shown)

There was no effect of the LID on maternal feed consumption in the prebreeding, gestation, or lactation periods. In the offspring, in the first 3 weeks after weaning, feed consumption in the LID groups was decreased and variable. For the first week measured, ages 28–35 days, feed consumption was decreased 34% in the NLID group and 44% in the CLID group. By the seventh week of life, 4 weeks after weaning, feed consumption was essentially equal across the three groups, and it remained so for the remainder of the study.

Body Weights

In the dams during the premating, gestation, and lactation periods, there were no significant differences in body weight or body weight gains between the controls and LID rats. Pup body weights were unaffected by iodine deficiency between 1 and 7 days of age. There was a numerical decrement (–9%) in the LID pups at the end of the second week of life, which became significant (–23%) by PND 21. At PND 28, the group mean body weights were decreased 27% and 36% in the NLID and CLID groups, respectively. These differences then steadily decreased until the NLID and CLID groups equaled controls by PND 77 and PND 98, respectively, and, by the end of the study, both LID groups numerically exceeded the controls (Fig. 2 and Table 2).

Terminal Body and Organ Weights

The dams were sacrificed after weaning their litters, approximately 5 months after arriving in the laboratory and starting on their respective diets. There was no significant body weight effect from the low iodine diet. LID group mean absolute thyroid weights, however, were increased 465%. Mean absolute kidney weights were significantly lower (–14.9%) in the LID group than in controls.

On PND 21, the mean body weight of sacrificed LID pups (42.0 \pm 4.5 g) was significantly less (–23.9%) than controls (55.2 \pm 5.3 g). Thyroid glands were markedly enlarged. The mean absolute thyroid weight of the LID pups (0.023 \pm .008 g) was 360% greater than controls (0.005 \pm 0.001 g); their relative weight was increased 450%. Notably, at this prepubertal stage, the LID group mean absolute and relative testes weights (0.128 \pm .018 g, 0.305 \pm 0.019 g/100) were markedly less than controls (–45.8% and –28.7%, respectively). LID mean relative brain weight (3.529 \pm 0.348 g/100) was significantly greater (+25.9%) than controls. The LID group mean absolute and relative kidney weights (0.435 \pm 0.055 g, 1.036 \pm 0.079 g/100) were 30.3% and 8.5% less than controls, respectively.

In the F1 males on the PND 133 \pm 1 termination, there were no statistically significant group differences in body weight (Table 1).

Sperm parameters subgroup. At PND 133 \pm 1, paired absolute testes weights in the NLID and CLID rats were numerically increased (+8.5% and +14.0%, respectively), but statistically identified only in the CLID group (Table 2). The

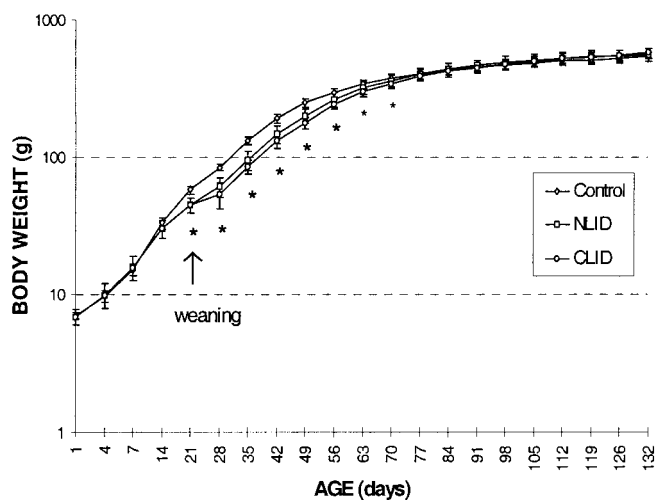


FIG. 2. In-life body weights of F1 male offspring. CLID, chronic low iodine diet; LID, low iodine diet; NLID, neonatal low iodine diet; PND, postnatal day. LID pup body weights fell below controls in their last week of nursing and remained lower for 6–8 weeks. Large asterisk indicates all LID pups (PND 21) or both NLID and CLID groups (PND 28–56) mean body weights are significantly less than controls. Small asterisk indicates only CLID mean body weight has significant decrement. Alpha = 0.05.

TABLE 2
The Effect of Neonatal Iodine Deficiency on Adult CD-1 Rat Testes Weights, with External Control Data from a Feed Restriction/Reproduction Study and a 13-Week Toxicology Study

Study/subgroup	Treatment	n	Body weight (g) ^a	Testes weight		DSP/testis 10E07
				(g) ^a	(g/100) ^a	
Sperm Parameters Subgroup	Control	9 ^b	517.2 ± 42.6	3.950 ± 0.283	0.767 ± 0.065	4.29 ± 0.9
	NLID	14 ^b	527.3 ± 50.3	4.286 ± 0.577	0.822 ± 0.107	4.80 ± 0.63
	CLID	10 ^b	562.5 ± 46.3	4.504 ± 0.467*	0.804 ± 0.087	5.05 ± 0.59
Testes Morphometry Subgroup	Control	5	525.7 ± 33.1	3.525 ± 0.250	0.672 ± 0.049	ND
	NLID	5	562.6 ± 77.0	4.533 ± 0.355**	0.815 ± 0.091**	ND
	CLID	5	529.3 ± 28.1	4.241 ± 0.284**	0.803 ± 0.070**	ND
External Controls: Feed Restriction study ^c	100% <i>ad libitum</i>	14	585.2 ± 49.5	3.636 ± 0.272	0.625 ± 0.063	3.87 ± 1.67
	90% <i>ad libitum</i>	13	579.9 ± 48.7	3.663 ± 0.346	0.632 ± 0.035	4.03 ± 1.08
	70% <i>ad libitum</i>	13	511.1 ± 27.6**	3.472 ± 0.418	0.680 ± 0.086	3.65 ± 0.80
	50% <i>ad libitum</i>	13	464.9 ± 34.7**	2.936 ± 0.239**	0.634 ± 0.057	3.25 ± 1.79**
13 Week tox. ^d	Control	10	525.9 ± 69.4	3.412 ± 0.209	0.654 ± 0.051	ND

Note. Neonatal iodine deficiency resulted in increased testes size and daily sperm production. CLID, chronic low iodine diet; DSP, daily sperm production; ND, not done; NLID, neonatal low iodine diet. The sperm parameters control group paired testes weight was unusually high compared to the morphometry group controls, and to CD rats used in a feed restriction study (147 days of age) and control CD rats from a 13-week toxicity study (≅137 days of age) that serve as concurrent and historical intralaboratory controls.

^a Values are ± one standard deviation.

^b n = 13 for NLID testes weights and DSP; one rat was excluded from this group for bilateral testes atrophy. Two control, 2 NLID, and 1 CLID rat in the sperm parameters group had unilateral testes atrophy; for these rats paired testes weights were calculated as 2× the weight of the normal testis.

^c Adapted from Carney *et al.*, 1998.

^d Report of the Dow Chemical Co., 1987.

* Statistically different from control mean by forced Wilcoxon's test, alpha = 0.05.

** Statistically different from control mean by Dunnett's test, alpha = 0.05.

mean testes weight of the control group (3.950 g) was, inexplicably, about 10% larger than our laboratory historical controls of the same approximate age, and similarly larger than the control littermates randomly assigned to the testes morphometry (perfused) subgroup. However, the heaviest individual control paired testes weight was 4.274 g, whereas there were several rats in the NLID and CLID groups with testes weights over 5 g (heaviest at 5.532 g), a size well outside the normal control range. Although testes weights in the NLID group were not statistically larger than controls ($p = 0.17$), 7 of the 13 rats had testes heavier than the heaviest testes among the control rats. (individual data not shown). Similarly, paired epididymal mean weights for the NLID and CLID groups (1.670 ± 0.132 and 1.732 ± 0.212 g, respectively) were numerically increased over controls (1.574 ± 0.177 g).

Within this subgroup, results were further complicated by six rats with spontaneous testicular atrophy unrelated to treatment, as explained below. Paired testis weights were calculated for those rats with unilateral atrophy by doubling the weight of the normal testis. We believe that this treatment of the data does not bias our results because unilateral testis atrophy is a late-onset disease (Wright, 1987), and compensatory hypertrophy following hemicastration occurs in rats only with neonatal castration (Cunningham *et al.*, 1978).

Brain weight relative to body weight was significantly

decreased (0.392 ± 0.031 g/100 g BW, -9.9%) in the CLID group at PND 133 ± 1 ; however, the CLID mean absolute brain weight (2.192 ± 0.101 g) was not significantly less than controls (2.235 ± 0.106 g). The mean thyroid gland weight in the CLID group (0.047 ± 0.008 g) was significantly increased (+81%) over controls (0.026 ± 0.005 g). Thyroid and brain weights of the NLID group were not different than controls, and there was no effect of the diet on kidney weights.

Testis morphometry subgroup. The control group mean testis weight (perfused) of 3.52 g, unlike the sperm parameter subgroup, was well within the expected range for CD rats of this age and weight (Table 2). The mean absolute testis weights of the NLID and CLID rats in this subgroup were significantly increased (+29% and +20%, respectively, vs controls); as were the relative testis weights (+21% and +19%, respectively). NLID and CLID epididymal weights (1.491 ± 0.345 and 1.490 ± 0.181 g, respectively) were numerically increased 16% in both LID groups over controls (1.284 ± 0.068). The CLID group mean absolute thyroid gland weight (0.049 ± 0.007) was significantly larger (+88%) than controls (0.026 ± 0.006 g); the NLID group mean thyroid weight was numerically larger (0.035 ± 0.006 , +35%). There was no significant effect of the diet on kidney or brain weight.

TABLE 3
Serum Thyroid Hormone Levels in Male Rats at 4, 21, 50 ± 1, and 133 ± 1 Days of Age

Age	Treatment	n	T4 (μg/dl)	T3 (ng/dl)
PND 4	Control	5	1.3 ± 0.7	ND
	LID	5	≤ 0.5*	ND
PND 21	Control	9	4.5 ± 1.2	124.4 ± 11.5
	LID	13	≤ 0.5**	58.8 ± 14.4**
PND 50 ± 1	Control	9	4.2 ± 1.0	94.0 ± 20.4
	NLID	10	4.6 ± 1.6	100.7 ± 19.4
	CLID	10	4.2 ± 1.5	69.2 ± 35.3
PND 133 ± 1	Control	9	5.1 ± 0.6	ND
	NLID	14	4.1 ± 1.1**	ND
	CLID	10	3.7 ± 1.1**	ND

Note. Iodine deficient neonatal rats were hypothyroid during the critical nursing period. CLID, chronic low iodine diet; LID, low iodine diet; ND, not done; NLID, neonatal low iodine diet; PND, postnatal day.

* Significantly different from control mean by Wilcoxon's test, alpha = 0.05.

** Significantly different from control mean by Dunnett's test, alpha = 0.05.

Thyroid Hormone Levels T4 and T3

(Table 3) At termination, immediately after weaning their pups, the control group dams had a significantly ($p < 0.05$) higher mean serum T4 ($2.9 \pm 1.3 \mu\text{g/dl}$) level than the LID group. None of the LID dams had detectable T4 (limit of detection $0.5 \mu\text{g/dl}$).

The LID pups sacrificed at PND 4 had undetectable T4, significantly less than control means. PND 4 blood samples had insufficient remaining volume for T3 analysis. At weaning (PND 21), T4 in the LID pups remained undetectable, whereas the control group mean T4 was $4.5 \pm 1.2 \mu\text{g/dl}$, and the mean T3 value for the LID pups was 53% lower than controls.

At PND 50 ± 1, there were no significant differences in serum T4 or T3 between controls and either the NLID or CLID groups. At the PND 133 ± 1 termination, however, both the NLID and CLID groups had T4 levels that were lower than controls ($p < 0.05$). However, the control T4 value ($5.1 \pm 0.6 \mu\text{g/dl}$) was unusually high, and the LID group means were near

published normals (Escobar del Rey *et al.*, 1987), suggesting that the PND 133 ± 1 mean decrements in the test groups were of doubtful biologic significance.

Pathology

All of the iodine-deficient dams and none of the controls had grossly enlarged and histologically hyperplastic thyroid glands. Additionally, two of the LID dams had small thyroid follicular adenomas.

All PND 21 LID male weanlings had grossly enlarged thyroid glands. At PND 133 ± 1, the increased size of the thyroid glands (see organ weight results above) of the CLID groups was, for most rats, not readily apparent on gross visual inspection. There were no other treatment-related gross findings. However, in the sperm parameters subgroup, there were six rats (two controls, three NLID, and one CLID) with unilateral (four right, one left) or bilateral (one) small and/or flaccid testes. Typically these testes were pale amber, slightly translucent and histologically severely atrophied: 90–100% of seminiferous tubules had "Sertoli only" morphology and a large amount of eosinophilic extratubular edema.

The unusually heavy testes of rats in the LID groups were histologically normal. The only treatment-related histologic finding was thyroid gland hyperplasia in all rats of the CLID group. No gross abnormalities were observed in the rats in the testis morphometry subgroup.

Testis Morphometry

(Table 4) The number of Sertoli cells (SC) per testis increased proportionately but nonsignificantly with testes weights in both the NLID (+24.8%) and CLID (+23.9%) groups. Round spermatids (RS) were significantly increased (20.4%) in the NLID group and numerically increased (15.8%) in the CLID group. The density of SC and RS had similar means across all treatment groups. As the testes of the iodine-deficient rats were, on average, larger than controls, the total number of Sertoli cells and round spermatids per pair of testes was directly related to the volume and weight of the testes.

TABLE 4
Histomorphometric Estimation of the Number of Sertoli Cells and Round Spermatids in the Testes of Control and Iodine Deficient Rats at 133 ± 1 Days of Age

n = 5	Paired testes weight (g)	SC/frame	Total SC (×10 ⁶)	RS/frame	Total RS (×10 ⁶)
Control	3.525 ± 0.250	0.84 ± 0.05	65.3 ± 4.3	6.17 ± 0.68	482.2 ± 47.7
NLID	4.553 ± 0.335*	0.81 ± 0.16	81.5 ± 19.0	5.81 ± 0.80	580.6 ± 66.4*
CLID	4.241 ± 0.284*	0.87 ± 0.16	80.9 ± 11.0	5.99 ± 0.97	558.2 ± 53.8

Note. Density of Sertoli cells and round spermatids per counting frame remained constant; total SC and RS varied with testes weight. CLID, chronic low iodine diet; NLID, neonatal low iodine diet; SC, Sertoli cells; RS, round spermatids.

* Statistically significant, $p < 0.05$, Dunnett's test.

Sperm Parameters

The NLID and CLID groups had DSP values that were 11.9% ($p = 0.23$) and 17.8% ($p = 0.07$) greater than concurrent controls (24.0% and 30.5% greater than historical controls, [Carney *et al.*, 1998]), respectively (Table 2). The findings closely paralleled the testes weight data, and therefore the values were nearly identical for spermatogenic efficiency across the three groups (data not shown).

Sperm Motility and Morphology

There were no treatment-related effects on percent motile or progressively motile sperm. There was a statistically identified increase in sperm with either an abnormal head or an abnormal tail in the NLID group; however, the CLID group was not significantly different than controls. The mean percent abnormal sperm for the control, NLID, and CLID groups were 7.4%, 14.0%, and 9.1%, respectively.

DISCUSSION

The LID caused hypothyroidism in the dams, and in their suckling offspring before and after the critical PND 4–16 period for Sertoli cell proliferation, as was documented by reduced T4 and T3 serum levels and goitrous thyroid enlargement in dams and offspring. At the more moderate level of iodine deficiency in the CLID group in the postweaning period, thyroid function was largely compensated by goiter, as evidenced by increased thyroid gland size and normal serum T4 levels. The NLID group recovered to normal thyroid hormone levels by PND 50 ± 1 , and near-normal thyroid gland size and normal morphology were demonstrated at termination.

The data confirmed that maternal and neonatal dietary iodine deficiency ultimately caused functional enlargement of the testes of male rat offspring. However, at weaning, the end of the critical period for inducing the hypothyroid testis effect, LID males had markedly retarded testes growth, a finding that emphasizes the delayed manifestation of testicular enlargement. Adult testes weights, DSP, and total Sertoli cell and round spermatid numbers all trended upward without exception and to a similar degree in both the NLID and CLID groups. However, statistical significance was less than uniform, primarily because of larger than expected testes in the control rats of the sperm parameters subgroup, in which the mean testes weight (3.950 g) was 14% larger than our laboratory historical controls of the same approximate age (Carney *et al.*, 1998), and similarly larger than the control group of littermates randomly assigned to the morphometry replicates (mean perfused testes weight 3.525 g). Statistically significant findings included increased absolute testes weight for the CLID rats in the sperm parameters subgroup, and, in the testes morphometry subgroup, increased absolute and relative testes weight for the CLID and NLID rats and increased total RS in the NLID rats. The interpretation that the nonstatistical increases in testes

weight and cell number were due to the LID derives largely from the incidence, only in the LID groups, of many rats with paired testis weights well above the historical normal range of individual values, some over 5 g. Within the morphometry subgroup, nonsignificant total Sertoli cell and round spermatid numbers had trends of similar magnitude and direction with all other parameters related to testes weight and sperm production.

Significantly, among functional sperm parameters there were no negative effects clearly attributable to the LID. Because no effect on sperm morphology was seen in the CLID rats, biologic significance for the statistically increased incidence of defective sperm in the NLID group is considered doubtful.

The only other apparent effect of the LID on either group of males at PND 133 ± 1 was smaller relative brain weights in the sperm parameters subgroup of CLID rats, a finding consistent with impaired brain development observed in severe iodine deficiency (Hetzel and Mano, 1989; Morreale de Escobar *et al.*, 1993). This finding is of doubtful significance in this case; the absolute weights were unaffected, and the finding was not replicated in the morphometry subgroup where relative brain weights across the three groups were nearly identical and the mean control brain weight was smaller than the statistically decreased CLID group in the sperm parameters subgroup.

In the dams, the primary effect of the LID was on the thyroid gland. Goiter was prominent, and there were small thyroid follicular epithelial cell adenomas in two rats. This is a common finding in rats undergoing chronic hyperstimulation by TSH, the normal physiologic response to hypothyroidism (Capen, 1997). For unknown reasons, the conception rate was low in both control and LID dams. However, the reproductive indices were apparently unaffected by iodine deficiency, with the single and important exception of litter size, which was significantly smaller in the LID dams.

The CLID pups we attempted to maintain on the original ≤ 20 ppb I diet began to die about a week after weaning, and we therefore added 40 ppb of iodine to their diet, after which no further unscheduled deaths occurred. This postweaning loss of pups was somewhat surprising, as the dams had been on the LID for 10 weeks prior to breeding, plus gestation and lactation, with no effect on survival. However, this occurrence served to emphasize both the relative sensitivity of younger rats to iodine deficiency, and the protection afforded the suckling pups by their mother's ability to concentrate iodine in milk, in contrast to the milk of humans or cows (Aquaron *et al.*, 1993; Delange *et al.*, 1988; Delange and Burgi, 1989; Escobar del Rey *et al.*, 1987). For this reason, the rat model may underestimate the consequences of comparable levels of dietary iodine deficiency in children.

Historically, the iodine deficient area of the United States and Canada, or "goiter belt," included the Dakotas, Minnesota, the adjacent prairie provinces, and the entire Great Lakes basin and St. Lawrence drainage. The Pacific Northwest was also affected (Markel, 1987). Mountainous, glaciated, and flood-

plain regions around the globe are leached of iodine. Prior to 1923, in some regions of Switzerland almost 100% of school children had large goiters (Burgi *et al.*, 1990), and in Michigan almost half of the children had palpable goiter (Markel, 1987). Iodination in the United States followed quickly after Switzerland, and in 1923 William J. Hale, a chemist at the Dow Chemical Company in Midland, Michigan, made the first iodized salt in America. Within a year it appeared on grocers' shelves and was quickly accepted by the public. In these areas endemic goiter soon disappeared (Markel, 1987).

Although there is no agreement that sperm counts have fallen, there is consensus that geographic differences exist. Additionally, there are regions, largely in the developing world but in parts of Europe as well, where the problem of iodine deficiency still exists in geographic pockets or where it has been addressed only in recent decades (Bleichrodt *et al.*, 1989; Delange *et al.*, 1986; Hetzel *et al.*, 1990). Thus, currently, there are otherwise reasonably homogeneous populations of reproductive-age men, who did or did not have the benefit of adequate iodine nutrition during their critical perinatal and childhood years. Data that could be revisited with this variable in mind include the Paris semen quality study (Auger *et al.*, 1995), which, when analyzed by year of birth between 1945 and 1962 for age-adjusted 30-year-olds, showed a decline from 102×10^6 to 51×10^6 sperm/ml. Iodized salt was made available in France in 1952 (Valeix *et al.*, 1994), the middle of the above period, suggesting Auger's data is consistent with our hypothesis. Others have noted the ongoing risk of iodine deficiency in parts of France (Gutekunst and Delange, 1992; Supersaxo *et al.*, 1991; Thieblot *et al.*, 1992). Comparing sperm counts and/or testes size versus childhood iodine status in these populations would be worthwhile.

An argument concerning the applicability of this model to humans is that thyroid goiter adequately compensates for moderate iodine deficiency. Consequently, one could argue that moderate deficiency does not cause significant hypothyroidism and therefore could not plausibly have an effect on the testis, or, at most, the effect would be present only in severe deficiency and would not be widespread enough to affect the sperm parameters of large populations. The normal T4 levels of the PND 50 ± 1 CLID rats in this study appear to support this argument. However, in populations of noncretin children from a number of countries with various degrees of iodine deficiency, there are reported significant negative effects on intelligence, psychomotor development (Bleichrodt *et al.*, 1996), and hearing acuity (Valeix *et al.*, 1994), suggesting that the subtle effects of less severe degrees of iodine deficiency are widespread in humans. Further, the temporal dynamics of Sertoli cell proliferation in the preadolescent human male are not well described, and its sensitivity to slight or episodic hypothyroidism has not been tested.

It appears that the basic thyroid hormone-mediated control of testis development is as applicable to humans as the other species in which the model has been demonstrated. In boys,

macroorchidism and very high sperm counts are associated with congenital hypothyroidism (Bruder *et al.*, 1995; Castro-Magana *et al.*, 1988), though these researchers and others (Meschede *et al.*, 1995; Nistal *et al.*, 1994) published in the human macroorchidism literature have not acknowledged the mechanism described in animals. Further, in rats, neonatal hyperthyroidism caused by daily injections of T3 results in smaller testes by the same mechanism, prematurely truncating Sertoli cell proliferation (van Haaster *et al.*, 1993), suggesting that the controlling effect of thyroid hormone on testis development may be continuous through the subnormal, normal, and supernormal range of hormone levels. Therefore, a measurable decline in the spermatogenic capacity of human populations caused by widespread and continuing improvements in juvenile iodine status appears to be quite plausible, and should be considered in any study designed to assess human semen quality on a global basis.

ACKNOWLEDGMENTS

This study was supported by a grant from the Chlorine Chemistry Council of the Chemical Manufacturers Association. The authors thank Tom Sanderson, Joni Pavlicek, Joyce Battjes, Usha Vedula, Wendy Kempf, Dean Dittenber, Lisa McFadden, Dave Myers, Sandy Eddy, and Kay Carnes for technical assistance.

REFERENCES

- Aquaron, R., Zarrouck, K., el Jarari, M., Ababou, R., Talibi, A., and Ardissonne, J. P. (1993). Endemic goiter in Morocco (Skoura-Toundoute areas in the high atlas). *J. Endocrinol. Invest.* **16**, 9–14.
- Auger, J., Kunstmann, J. M., Czyglik, F. C., and Jouannet, P. (1995). Decline in semen quality among fertile men in Paris during the past 20 years. *N. Eng. J. Med.* **332**, 281–285.
- Bleichrodt, N., Escobar del Rey, F., Morreale de Escobar, G., Garcia, I., and Rubio, C. (1989). Iodine deficiency, implications for mental and psychomotor development in children. In *Iodine and the Brain* (G. R. DeLong, J. Robbins, and P. G. Condliffe, Eds.), pp. 269–287. Plenum Press, New York.
- Bleichrodt, N., Shrestha, R. M., West, C. E., Hautvast, J. G. A. J., van de Vijver, F. J. R., and Born, M. P. (1996). The benefits of adequate iodine intake. *Nutr. Rev.* **54**, s72–s78.
- Bonde, J. P. E., Kold Jensen, T., Brixen Larsen, S., Abell, A., Scheike, T., Hjollund, N. H. I., Kolstad, H. A., Ernst, E., Giwercman, A., Skakkebaek, N. E., Keiding, N., and Olsen, J. (1998). Year of birth and sperm count in 10 Danish occupational studies. *Scand. J. Environ. Health* **24**, 407–413.
- Brake, A., and Krause, W. (1992). Decreasing quality of semen [letter]. *Brit. Med. J.* **305**, 1498.
- Bruder, J. M., Samuels, M. H., Bremner, W. J., Ridgway, E. C., and Wierman, M. E. (1995). Hypothyroidism-induced macroorchidism: Use of a gonadotropin-releasing hormone agonist to understand its mechanism and augment adult stature. *J. Clin. Endocrinol. Metab.* **80**, 11–16.
- Burgi, H., Supersaxo, Z., and Selz, B. (1990). Iodine deficiency diseases in Switzerland one hundred years after Theodor Kocher's survey: A historical review with some new goitre prevalence data. *Acta. Endocrin. (Copenh)* **123**, 577–590.
- Capen, C. C. (1997). Mechanistic data and risk assessment of selected toxic end points of the thyroid gland. *Toxicol. Pathol.* **25**, 39–48.
- Carlsen, E., Giwercman, A., Keiding, N., and Skakkebaek, N. E. (1992).

- Evidence for decreasing quality of semen during the past 50 years. *Brit. Med. J.* **305**, 609–613.
- Carney, E. W., Scortichini, B., and Crissman, J. W. (1998). Feed restriction during *in utero* and neonatal life: Effects on reproductive and developmental end points in the CD rat. *Toxicologist* **42**, 505–506.
- Castro-Magana, M., Angulo, M., Canas, A., Sharp, A., and Fuentes, B. (1988). Hypothalamic-pituitary gonadal axis in boys with primary hypothyroidism and macroorchidism. *J. Pediatr.* **112**, 397–402.
- Clermont, Y., and Harvey, S. C. (1965). Duration of the cycle of the seminiferous epithelium of normal, hypophysectomized and hypophysectomized-hormone treated albino rats. *Endocrinology* **76**, 80–89.
- Cooke, P. S. (1991). Thyroid hormone and testis development: A model system for increasing testis growth and sperm production. *Ann. N.Y. Acad. Sci.* **637**, 122–132.
- Cooke, P. S., Hess, R. A., Porcelli, J., and Meisami, E. (1991). Increased sperm production in adult rats following transient neonatal hypothyroidism. *Endocrinology* **129**, 244–248.
- Cooke, P. S., Kirby, J. D., and Porcelli, J. (1993). Increased testes growth and sperm production in adult rats following transient neonatal goitrogen treatment: Optimization of the propylthiouracil dose and effects of methimazole. *J. Reprod. Fertil.* **97**, 493–499.
- Cooke, P. S., and Meisami, E. (1991). Early hypothyroidism in rats increases adult testis and reproductive organ size but does not change testosterone levels. *Endocrinology* **129**, 237–243.
- Cooke, P. S., Zhao, Y., and Hansen, L. G. (1995). Neonatal polychlorinated biphenyl (PCB) treatment increases adult testis size and sperm production in the rat. *Toxicol. Appl. Pharmacol.* **136**, 112–117.
- Cunningham, G. R., Tindall, D. J., Huckins, C., and Means, A. R. (1978). Mechanisms for the testicular hypertrophy which follows hemicastration. *Endocrinology* **102**, 16–23.
- Delange, F., Bourdoux, P., Chanoine, J. P., and Ermans, A. M. (1988). Physiopathology of iodine nutrition during pregnancy, lactation, and early postnatal life. In *Vitamins and Minerals in Pregnancy and Lactation* (H. Berger, Ed.), Nestle Nutrition Wkshp Series. **16**, 205–214.
- Delange, F., and Burgi, H. (1989). Iodine deficiency disorders in Europe. *Bull. World Health. Org.* **67**, 317–325.
- Delange, F., Heidemann, P., Bourdoux, P., Larsson, A., Vigneri, R., Klett, M., Beckers, C., and Stubbe, P. (1986). Regional variations of iodine nutrition and thyroid function during the neonatal period in Europe. *Biol. Neonate* **49**, 322–330.
- Escobar del Rey, F., Mallol, J., Pastor, R., and Morreale de Escobar, G. (1987). Effects of maternal iodine deficiency on thyroid hormone economy of lactating dams and pups: Maintenance of normal cerebral 3,5,3'-triiodo-L-thyronine concentrations in pups during major phases of brain development. *Endocrinology* **121**, 803–811.
- Filler, R. (1993). Methods for evaluation of rat epididymal sperm morphology. In *Methods in Toxicology* (R. E. Chapin and J. J. Heindel, Eds.), Vol. 3, Part A, Male Reproductive Toxicology. Academic Press, San Diego.
- Fisch, H., and Goluboff, E. T. (1996). Geographic variations in sperm counts: A potential cause of bias in studies of semen quality. *Fertil. Steril.* **65**, 1044–1046.
- Fisch, H., Goluboff, E. T., Olson, J. H., Feldshuh, J., Broder, S. J., and Barad, D. H. (1996). Semen analyses in 1,283 men from the United States over a 25-year period: No decline in quality. *Fertil. Steril.* **65**, 1009–1014.
- Gutekunst, R., and Delange, F. (1992). Assessment techniques and the epidemiology of iodine deficiency disorders in Europe. *Eur. J. Intern. Med.* **3**(suppl), 71–75.
- Hess, R. A., Bunick, D., Lee, K.-H., Bahr, J., Taylor, J. A., Korach, K. S., and Lubahn, D. B. (1997). A role for oestrogens in the male reproductive system. *Nature* **390**, 509–512.
- Hess, R. A., Cooke, P. S., Bunick, D., and Kirby, J. D. (1993). Adult testicular enlargement induced by neonatal hypothyroidism is accompanied by increased Sertoli and germ cell numbers. *Endocrinology* **132**, 2607–2613.
- Hetzel, B. S., and Mano, M. T. (1989). A review of experimental studies of iodine deficiency during fetal development. *J. Nutr.* **119**, 145–151.
- Hetzel, B. S., Potter, B. J., and Dulberg, E. M. (1990). The iodine deficiency disorders: Nature, Pathogenesis and Epidemiology. In *Aspects of Some Vitamins, Minerals and Enzymes in Health and Disease* (G. H. Bourne, Ed.), Vol. 62, pp. 59–119. World Rev. Nutr. Diet., Karger, Basel.
- Jannini, E. A., Olivieri, S., Francavilla, S., Gulino, A., Ziparo, E., and D'Armiato, M. (1990). Ontogenesis of the nuclear 3,5,3'-triiodothyronine receptor in the male rat testis. *Endocrinology* **126**, 2521–2526.
- Janssen, K. P. L. T. M. K., van der Heide, D., Visser, T. J., Kaptein, E., and Beynen, A. C. (1994). Thyroid function and deiodinase activities in rats with marginal iodine deficiency. *Biol. Trace Elem. Res.* **40**, 237–246.
- Joyce, K. L., Porcelli, J., Cooke, P. S. (1993). Neonatal goitrogen treatment increases adult testis size and sperm production in the mouse. *J. Androl.* **14**, 448–455.
- Kirby, J. D., Cooke, P. S., Arambepola, N., Bunick, D., Hess, R. A., and Turek, F. W. (1993). Neonatal hypothyroidism increases adult testis size and sperm production in the hamster (*Mesocricetus auratus*). *Program of the XIIth North American Testis Workshop*, Tampa, FL, p. 51 (abstract).
- Kirby, J. D., and Mankar, M. V. (1995). Adult testicular enlargement in broiler breeder males following transient prepubertal propylthiouracil (PTU) treatment. *Poult. Sci.* **74**(suppl 1), 43.
- Markel, H. (1987). "When it rains it pours": endemic goiter, iodized salt, and David Murray Cowie, MD. *Am. J. Publ. Health* **77**, 219–229.
- Meachem, S. J., McLachlan, R. I., de Kretser, D. M., Robertson, D. M., and Wreford, N. G. (1996). Neonatal exposure of rats to recombinant follicle stimulating hormone increases adult Sertoli and spermatogenic cell numbers. *Biol. Reprod.* **54**, 36–44.
- Meschede, D., Behre, H. M., and Nieschlag, E. (1995). Endocrine and spermatological characteristics of 135 patients with bilateral megalotestis. *Andrologia* **27**, 207–212.
- Morreale de Escobar, G., Obregon, M. J., Calvo, R., and Escobar del Rey, F. (1993). Feto-maternal thyroid hormone relationships in iodine deficiency: An experimental approach. In *Iodine Deficiency in Europe* (F. Delange et al., Eds.), pp. 171–180, Plenum Press, New York.
- Nistal, M., Martinez-Garcia, F., Regadera, J., Cobo, P., and Paniagua, R. (1994). Macro-orchidism: A clinicopathological approach. *J. Urol.* **151**, 1155–1161.
- Olsen, G. W., Bodner, K. M., Ramlow, J. M., Ross, C. E., and Lipshultz, L. I. (1995). Have sperm counts been reduced 50% in 50 years? A statistical model revisited. *Fertil. Steril.* **63**, 887–893.
- Paulsen, C. A., Berman, N. G., and Wang, C. (1996). Data from men in greater Seattle area reveals no downward trend in semen quality: Further evidence that deterioration of semen quality is not geographically uniform. *Fertil. Steril.* **65**, 1015–1020.
- Robb, G. W., Amann, R. P., and Killian, G. J. (1978). Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J. Reprod. Fertil.* **54**, 103–107.
- Saidi, J. A., Chang, D. T., Goluboff, E. T., Bagiella, E., Olsen, G., and Fisch, H. (1999). Declining sperm counts in the United States? A critical review. *J. Urol.* **161**, 460–462.
- Seed, J., Chapin, R. E., Clegg, E. D., Dostal, L. A., Foote, R. H., Hurtt, M. E., Klinefelter, G. R., Makris, S. L., Perreault, S. D., Schrader, S., Seyler, D., Sprando, R., Treinen, K. A., Veeramachaneni, D. N. R., and Wise, L. D. (1996). Methods for assessing sperm motility, morphology and counts in the rat, rabbit, and dog: A consensus report. *Reprod. Toxicol.* **10**, 237–244.
- Sherins, R. J. (1995). Are semen quality and male fertility changing? *N. Eng. J. Med.* **332**, 327–328.

- Supersaxo, Z., Selz, B., Hasler, P., Wespi, H. J., Abelin, T., and Burgi, H. (1991). Is iodization of salt still necessary? Recent data on iodine supply in Switzerland. *Schweiz. Med. Wochensch.* **121**, 317–323.
- Swan, S. H., Elkins, E. P., and Fenster, L. (1997). Have sperm densities declined? A reanalysis of global trend data. *Environ. Health Perspect.* **105**, 1228–1232.
- Thieblot, P., Samrani, J., and Tauveron, I. (1992). Epidemiologie du goitre en Auvergne. *Ann. Endocrinol. (Paris)* **53**, 107–111.
- Tjoa, W. S., Smolensky, P. H., Hsi, B. P., Steinberger, E., and Smith, K.D. (1982). Circannual rhythm in human sperm count revealed by serially independent sampling. *Fertil. Steril.* **38**, 454–459.
- Toth, G. P., Wang, S.-R., McCarthy, H., Tocco, D. R., and Smith, M. K. (1992). Effects of three male reproductive toxicants on rat cauda epididymal sperm motion. *Reprod. Toxicol.* **6**, 507–515.
- Valeix, P., Preziosi, P., Rossignol, C., Farnier, M. A., and Hercberg, S. (1994). Relationship between urinary iodine concentration and hearing capacity in children. *Eur. J. Clin. Nutr.* **48**, 54–59.
- van Haaster, L. H., De Jong, F. H., Docter, R., and De Rooij, D. G. (1992). The effect of hypothyroidism on Sertoli cell proliferation and differentiation and hormone levels during testicular development in the rat. *Endocrinology* **131**, 1574–1576.
- van Haaster, L. H., De Jong, F. H., Docter, R., and De Rooij, D. G. (1993). High neonatal triiodothyronine levels reduce the period of Sertoli cell proliferation and accelerate tubular lumen formation the rat testis, and increase serum inhibin levels. *Endocrinology* **133**, 755–760.
- Wittmaack, F. M., and Shapiro, S. S. (1992). Longitudinal study of semen quality in Wisconsin men over one decade. *Wis. Med. J.* **91**, 477–479.
- Wreford, N. G. (1995). Theory and practice of stereological techniques applied to the estimation of cell number and nuclear volume in the testis. *Microsc. Res. Tech.* **32**, 423–436.
- Wright, J. R., Jr. (1987). Atrophy, Testis, Rat. In *Genital System, Monographs on Pathology of Laboratory Animals, Sponsored by the International Life Sciences Institute* (T. C. Jones, U. Mohr, and R. D. Hunt, Eds.), pp. 218–225. Springer-Verlag, New York.