Fertility in Rats after Long-Term Jojoba Meal Supplementation

Marnix M. Cokelaere,*,† Johan Buyse,‡ Paul Daenens,§ Eddy Decuypere,‡ Eduard Kühn,∥ and Maurits Van Boven§

Interdisciplinary Research Center, Katholieke Universiteit Leuven, Campus Kortrijk, Universitaire Campus, E. Sabbelaan, B-8500 Kortrijk, Belgium, Laboratory of Physiology and Immunology of Domestic Animals, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Heverlee, Belgium, Laboratory of Toxicology, Katholieke Universiteit Leuven, Van Evenstraat 4, B-3000 Leuven, Belgium, and Laboratory of Comparative Endocrinology, Katholieke Universiteit Leuven, Naamse Straat 61, B-3000 Leuven, Belgium

During 11 weeks, weanling rats received either normal food or food supplemented with 3% deoiled jojoba meal or were pair-fed to the jojoba meal treated group. Afterward, control females (CF) were mated either with control males (CM), males taking jojoba-supplemented food (JJM), or males pair-fed to JJM (PFM). Jojoba-treated females (JJF) and females pair-fed to JJF (PFF) were mated with CM. The food regimen was continued during gestation. At day 15 of gestation, several fertility parameters were measured. In JJF and PFF a decrease in the number of corpora lutea and of living fetuses was observed, provoked by a reduced food intake before conception. An excess in fetal body weight reduction in JJF was explained by protein shortage during gestation and is paralleled by decreased maternal plasma progestagens and increased thyroid hormone (T3) concentrations. In males, no effect on these fertility parameters has been observed.

INTRODUCTION

The jojoba shrub (*Simmondsia chinensis*) produces oil-containing nuts. After oil extraction, a protein-rich meal remains which is difficult to use as animal feed because of the presence of glycosides, with simmondsin and simmondsin 2′-ferulate as the most important components (Elliger et al., 1973, 1974). Simmondsin and its analogues inhibit food intake (Booth et al., 1974; Cokelaere et al., 1992a,b). In adult rats, food intake inhibition causes the same emaciation as in rats pair-fed to rats treated with pure simmondsin (Cokelaere et al., 1992a,b). In young rats, the food intake inhibition caused by supplementation of food with deoiled jojoba meal induces a more pronounced growth retardation than in pair-fed rats. This has been ascribed to the induction of a relative protein shortage in growing animals, due to the presence of tannins and trypsin inhibitors in jojoba meal, which induces an increase in plasma thyroid hormone concentration with concomitant energy dissipation and a lower food efficiency (Cokelaere et al., 1993). Some authors presume that simmondsins are toxic (Booth et al., 1974; Verbiscar et al., 1980). According to Williams (1980), HCN could be liberated during the metabolism of simmondsin in mice, although this could not be demonstrated in rats (Cokelaere et al., 1992b). Several experiments have demonstrated a pronounced decrease of fertility after jojoba supplementation in rats (Booth et al., 1974; Weber and Reid, 1975; Verbiscar and Banigan, 1982). However, no pair-fed studies have been conducted to compare the influence of caloric or protein deficiency in prepubertal life or during gestation. Both deficiencies are known to decrease fertility in rats (Horn, 1955; Ghafoorunissa, 1980; Lintern-Moore et al., 1981; Ahokas et al., 1983; Snoeck et al., 1990). The following experiments aim to discriminate between the influences of food intake reduction and possible supplemental effects of jojoba meal on fertility in male and female rats. Concomitantly, progesterone and estradiol were measured at day 15 of pregnancy as well as thyroid hormones, to detect possible effects of protein shortage in animals treated with jojoba meal.

EXPERIMENTAL DESIGN

Eighty-six weanling Wistar rats (56 females and 30 males) were maintained under normal laboratory circumstances (22 °C, light from 8 a.m. to 8 p.m., relative humidity 40–60%, water ad libitum). The animals were divided into six groups. (a) 34 females received normal laboratory mash [control females (CF)]. (b) 10 females received normal laboratory mash supplemented with 3% deoiled jojoba meal [jojoba females (JJF)]. (c) 12 females were pair-fed to JJF [pair-fed females (PFF)]. (d) 10 males received normal laboratory mash [control males (CM)]. (e) 10 males received normal laboratory mash supplemented with 3% deoiled jojoba meal [jojoba males (JJM)]. (f) 10 males were pair-fed to JJM [pair-fed males (PFM)]. Their food uptakes and weight gains were measured twice a week. The deoiled jojoba meal was obtained by Soxhlet extraction of press-cake with *n*-hexane for 8 h. The concentrations of simmondsin and simmondsin 2′-ferulate in deoiled jojoba meal were 3.6 and 0.7%, respectively. The analytical method has been described previously (Cokelaere et al., 1993). Eleven weeks after the start of the experiment, a vaginal smear was taken from every female at 5 p.m. Animals in pre-estrus or estrus were caged separately overnight with a male, according to the following mating outline: CF × CM, CF × JJM, CF × PFM, JJF × CM, PFF × CM. Every CM was used three times for CF, JJM, and PFF. Coitus was determined by inspection of vaginal smears the following day at 8 a.m. Animals with a negative smear were allowed to mate in a following estrus cycle. All animals had positive smears after a second trial. At day 15 of gestation (day 0 = day of positive vaginal smear) the females were killed by CO_2 asphyxiation. Blood was obtained by heart puncture to determine the plasma concentrations of the thyroid hormones triiodothyronine (T3) and tetraiodothyronine (T4), estrogens, and progestagens. T3 and T4 concentrations were determined with radio immunoassay (RIA) (antisera and standards obtained from Byk-Sangtec, Germany) (Detection limits, for T3, 0.063–8 ng/mL; for T4, 0.39–100 ng/mL. Intra- and inter-assay coefficients of variation were 2 and 8% for T3 and 2.7 and 4% for T4, respectively.) Concentrations of estrogens (E_2) and progestagens (P_4) were determined using a RIA kit (Biotecx Laboratories Inc., Friendswood, TX). The purity of

† Interdisciplinary Research Center.
‡ Laboratory of Physiology and Immunology of Domestic Animals.
§ Laboratory of Toxicology.
∥ Laboratory of Comparative Endocrinology.

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Table I. Body Weight (BW) Evolution of Female Rats during 15 Days of Pregnancy[a]

	start	day 15	difference in % of BW
CF × CM	243[a] ± 9	294[a] ± 6	20.8[a] ± 1.3
CF × JJM	233[a] ± 6	277[a] ± 3	18.9[a] ± 0.9
CF × PFM	234[a] ± 3	278[a] ± 6	18.6[a] ± 1.2
JJF × CM	184[b] ± 4	207[b] ± 3	12.5[b] ± 0.3
PFF × CM	219[c] ± 3	241[c] ± 4	10.04[b] ± 0.5

[a] Abbreviations, see text. Mean ± SEM. Anova followed by t-test. Different indices indicate significantly different values ($p < 0.05$).

the antigen and the specificity of the antisera were guaranteed by the supplier. The intra- and interassay coefficients of variation were 4.8 and 6% for the P_4 RIA and 5.5 and 6.5% for the E_2 RIA, respectively. The ovaries were removed to count the number of corpora lutea (CL). Living fetuses were removed from the uterus, uncovered from embryonic membranes, dabbed dry, weighed, and inspected under a stereomicroscope for gross malformations. Resorptions (R) were calculated by subtracting the number of living fetuses from the number of CL. Runts were determined as fetuses with a body weight (BW) 25% below average weight of littermates. Statistical analysis of data was done by ANOVA followed by unpaired one-tailed t-test or X^2-tests for proportional data.

RESULTS AND DISCUSSION

Compared to CM, supplementation of the food with 3% deoiled jojoba meal causes a decrease in food uptake in JJM of about 20.1% (± 0.8) with a maximum of 26.3% in week 2 and gradually slowing to 17.1% in week 10. In JJF similar results were obtained with a mean reduction of food uptake of about 22.9% (± 0.9) and with a maximum of 33.4% in week 3, gradually slowing to 19.4% in week 11. These results are in the same range as those described in a previous paper on growth patterns in weanling male rats after supplementation with 3% deoiled jojoba meal (Cokelaere et al., 1993). The BWs in week 11 were, respectively, 378 g (± 5 g) for CM, 269 g (± 6 g) for JJM, 309 g (± 7 g) for PFM, 238 g (± 7 g) for CF, 184 g (± 4 g) for JJF, and 219 g (± 3 g) for PFF. The differences in BW between JJ and PF animals may be caused by a relative protein shortage in the jojoba-supplemented rations, due to the presence of tannins and trypsin inhibitors (Cokelaere et al., 1993).

During gestation, the weight increase of CF was about twice that of JJF or PFF. The pretreatment of the males had no influence on that parameter. There was no difference in weight gain, expressed in percent of BW, between JJF and PFF (Table I).

Table II summarizes the results of the fertility parameters examined. At autopsy, almost all females were pregnant. The differences in proportion of pregnant females after controlled mating between the different groups were not statistically significant. The ovaries of the nonfertilized females contained normal maturing follicles. Compared to CF, a similar decrease in the number of CL in both JJF and PFF was observed. Restricted food uptake is known to reduce the ovarian follicle population of the prepubertal Wistar female (Lintern-Moore et al., 1981). Therefore, it is concluded that the decrease in CL in JJF is due to food restriction before gestation.

The number of living fetuses was decreased to the same extent in both JJF and PFF. The number of resorptions of fetuses after conception was the same for all groups studied. It is concluded that the lower number of living fetuses is entirely caused by the decreased number of mature ovarian follicles, which is caused by food restriction before conception. The reduction of living fetuses is about 24% and fits well with the reduction in litter size as described by Weber and Reid (1975) after 4 weeks of 5% jojoba meal supplementation in female mice. The authors explained the reduction in litter size as a result of toxic factors in jojoba meal. Our results suggest that their conclusion is probably not justified. The presence of anovulatory ovaries observed by Verbiscar et al. (1982) after chronic oral administration of simmondsin can probably be explained by the food intake inhibition with concomitant weight loss in female rats, but pair-fed studies should be done to exclude toxic effects of simmondsin itself on this parameter.

No influence of the treatment on the number or frequency of runts in JJF was observed. This strengthens the hypothesis that there is no liberation of HCN during metabolism of simmondsin in rats (Cokelaere et al., 1992b). If HCN should be liberated, an increased number and frequency of runts should be observed, as is described in hamsters fed cyanide-containing food (Frakes et al., 1986).

Gross anatomical malformations of the fetuses were absent on stereomicroscopic inspection, but further investigations are needed to exclude teratogenic influences of jojoba meal, although this is rather unlikely in view of the number of runts and the fetal BWs.

Fetal BWs were reduced in JJF and PFF, but this was somewhat more pronounced in JJF. A reduction in fetal BW is known to occur in female rats with reduced food intake (Ahokas et al., 1983). It is concluded that the reduction in fetal BW in PFF is due to the reduced food intake. In JJF the excess in fetal BW reduction must be caused by a supplemental effect of the jojoba meal.

Table III shows the plasma T3 and T4 concentrations during gestation. T3 concentrations were increased in JJF compared to CF. In JJF, the T3/T4 ratio was increased. A similar phenomenon has been described in actively growing male rats receiving food supplemented with 3% deoiled jojoba meal. It has been argued that the T3 increase could be due to a relative protein shortage in actively growing rats, induced by the tannins and trypsin inhibitors and possibly simmondsins of jojoba meal which could liberate cholecystokinin (CCK). CCK can induce excessive protein loss by stimulating the enzyme secretion of the pancreas (Cokelaere et al., 1993). Buyse et al. (1992) also have shown that growing meat-type chickens fed a low dietary protein content had higher plasma T3 concentrations than chickens fed a normal protein diet. In adult rats with a lower protein requirement, 3% jojoba meal in the rations should be unable to induce a protein shortage by the mechanisms discussed above (Cokelaere et al., 1993). During gestation, however, the need for protein increases again. So the supplemention with 3% jojoba meal can induce a relative protein shortage with a concomitant increase of T3. The concentrations of estrogens and progestagens are summarized in Table IV. There were no statistically significant differences between the experimental groups for the estrogens. The concentration of progestagens has a tendency to decrease in JJF with a marginally nonsignificant difference ($p = 0.055$). A decrease of maternal plasma progesterone concentration is known to occur in pregnant rats under protein malnutrition (Mulay et al., 1982). An unexplained increase in progestagen concentration has been observed in CF × JJM. The observation of both increased T3 and decreased progestagen concentrations strengthens our hypothesis that jojoba meal supplementation induces not only a food intake inhibition but also a relative protein shortage. Protein shortage is known to reduce fetal BW in rats (Frakes et al., 1986; Snoeck et al., 1990). It therefore is concluded that the relative protein shortage induced by jojoba supplementation can explain the lower BW of the

Table II. Effect of 3% Jojoba Meal Supplementation on Female and Male Fertility[a]

	FF	CL	F	res	runts	no. of litters with runts	fetal BW, g
CF × CM (n = 12)	11	14.7[a] ± 0.4	11.9[a] ± 0.8	2.8[a] ± 0.7	3/131[a]	3/11[a]	148[a] ± 14
CF × JJM (n = 10)	10	13.3[a] ± 0.5	10.5[a] ± 0.8	2.8[a] ± 0.7	4/104[a]	2/10[a]	145[a] ± 2
CF × PFM (n = 12)	12	13.8[a] ± 0.4	11.0[a] ± 0.4	2.8[a] ± 0.4	1/132[a]	1/12[a]	143[a] ± 1
JJF × CM (n = 10)	8	11.8[b] ± 0.6	8.9[b] ± 1.2	2.9[a] ± 0.9	2/71[a]	1/8[a]	121[b] ± 2
PFF × CM (n = 12)	12	12.7[b] ± 0.3	10.2[ab] ± 0.5	2.5[a] ± 0.6	9/122[a]	8/12[b]	129[c] ± 2

[a] Results on day 15 of pregnancy. FF, females containing fetuses; CL, corpora lutea; F, number of living fetuses; res, resorptions (CL minus F); other abbreviations, see text. Mean ± SEM. Anova followed by t-test or X^2-test for proportions. Different indices indicate significantly different values ($p < 0.05$).

Table III. Plasma T3 and T4 Concentrations (Nanograms per Milliliter) in Female Rats on Day 15 of Pregnancy[a]

	T4	T3	T3/T4 × 100
CF × CM (n = 12)	19.53[a] ± 0.74	0.416[a] ± 0.082	2.182[a] ± 0.150
CF × JJM (n = 10)	20.71[a] ± 0.83	0.460[a] ± 0.022	2.241[a] ± 0.116
CF × PFM (n = 12)	20.57[a] ± 0.74	0.448[a] ± 0.027	2.195[a] ± 0.142
JJF × CM (n = 10)	19.45[a] ± 1.54	0.573[b] ± 0.035	3.469[b] ± 0.287
PFF × CM (n = 12)	21.28[a] ± 1.00	0.398[a] ± 0.023	1.892[a] ± 0.126

[a] Abbreviations, see text. Mean ± SEM. Anova followed by t-test. Different indices indicate significantly different values ($p < 0.05$).

Table IV. Plasma Concentrations of Estrogen (E_2) and Progestagen (P_4) in Female Rats at Day 15 of Pregnancy[a]

	estrogen, pg/mL	progestagen, ng/mL
CF × CM (n = 12)	20.4[a] ± 3.5	19.4[a] ± 4.6
CF × JJM (n = 10)	13.7[a] ± 1.2	41.0[b] ± 6.6
CF × PFM (n = 12)	16.0[a] ± 1.1	28.8[a] ± 6.1
JJF × CM (n = 10)	17.5[a] ± 3.4	9.39[a] ± 3.0
PFF × CM (n = 12)	16.1[a] ± 1.7	21.5[a] ± 7.9

[a] Abbreviations, see text. Mean ± SEM. Anova followed by t-test. Different indices indicate significantly different values ($p < 0.05$).

fetuses in JJF compared to PFF. Concerning the fertility parameters investigated in male rats in this experiment, no influence of 3% jojoba meal supplementation has been observed. In previous investigations, no influence of prolonged supplementation with 3% jojoba meal on the relative weights of testis, seminal vesicles, or prostate has been seen (Cokelaere et al., 1993). This seems in contradiction to the results of Booth et al. (1974). However, it is not clear whether these authors used deoiled meal or not, and they did not indicate the proportion of jojoba meal in the diet or the degree of food intake inhibition provoked by the meal, nor did they indicate how long the animals consumed the meal. Jojoba oil is indigestible and provokes diarrhea with a supplemental loss of nutrients (Verbiscar et al., 1980) and edema of the testis (Booth, 1972). The degree of food restriction also is important. A food restriction of 20% for 12 weeks in weanling male Wistar rats had no visible effects on the male reproductive organs and fertility (Ghafoorunissa, 1980). However, weanling rats fed a diet low in protein for 30 days showed a reduction in food intake of about 60% and a cessation of spermatogenesis (Horn, 1955). Ten percent of nondeoiled meal, as used by Booth et al. (1974), provokes a food intake reduction of about 80%. Probably the results of Booth et al. (1974) can be explained by a very low food and protein uptake.

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