Relationship between testicular blood flow, testosterone secretion and spermatogenic activity in young and adult wild red foxes (*Vulpes vulpes*)

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Summary. Testicular capillary blood flow (TCBF) was measured by the radioactive inert gas clearance technique throughout the reproductive life of young adult foxes and was related to the spermatogenic and androgenic activities of the testis. Mean ($\pm$ S.E.M.) blood flow ($\text{ml min}^{-1} \text{g}^{-1}$) was maximal in January in adults during the mating period (0.65 ± 0.03), and in pubertal animals (0.62 ± 0.04). At this time spermatozoa were observed in the testes of all animals, but testicular weight and circulating testosterone levels were lower in the pubescent foxes than in the adults. TCBF was minimal during immaturity (0.29 ± 0.03) and during the resting period of the adult (0.12 ± 0.01). These values were associated with a low testosterone level and with the multiplication of gonocytes in the young or with the seasonal very low spermatogenic activity in the adult. During the prepubertal period, TCBF slowly increased and was accompanied by testicular growth. In the adult, in September, TCBF rapidly increased without changes of testicular size and then slowly increased as the testes enlarged. High plasma testosterone concentrations occurred later. During the period of testicular regression, TCBF, testicular size, spermatogenic and androgenic activities decreased together.

Introduction

The fox is a seasonal breeder and the changes in the reproductive organs of young and adult male foxes have been described (Rowlands & Parkes, 1935; Creed, 1960; Fairley, 1970; Joffre, 1976a). The young are born in March, the prepubertal period starts in October, and puberty occurs in January at the end of the first year of life. The adult fox has a well defined breeding season, extending from December to February, after which the testes rapidly regress and remain quiescent from May to September. Testicular blood flow has been studied (see Setchell, 1970), but its relationship with spermatogenesis or hormonal secretion is poorly understood. In the present study, testicular capillary blood flow (TCBF) was determined in foxes and attempts were made to relate the pattern of TCBF to testicular weight and to the spermatogenic activities of the testis.

Materials and Methods

Foxes (*Vulpes vulpes*) were caught in south-western and mid-western France and were individually caged under normal daylight and temperature conditions. The animals were fed with dead chickens and were allowed free access to water. The foxes lived uneventfully under these conditions; no important differences in the reproductive activity between wild foxes and those reared in captivity have been observed (Joffre, 1976a).

Testicular capillary blood flow was measured by a radioactive inert gas clearance technique (Joffre, M. & Joffre, J., 1971; Joffre, J. & Joffre, M., 1973) on 96 foxes, 46 of which were immature or prepubertal. They were anaesthetized with an intraperitoneal injection of sodium barbital (15 mg/kg), and xenon-133 ($\text{Xe } 133\text{-S}_1$; I.R.E.Belgium; sp. act. 70–100 mCi/ml) in saline solution (0.01–0.02 ml) was injected directly into the testis through the scrotum with an intradermal needle (29 gauge). Foxes were used at approximately monthly intervals between 1 and 24 months of age, and they were then castrated to check the spermatogenic state of the testis histologically.

In these conditions, the clearance curves showed two components: a fast one, which has been previously shown to be related to testicular blood flow, and a slow one representing blood flow in the epididymal fat (Joffre, M. & Joffre, J., 1971). Since the partition coefficient of ^{133}Xe between the testis and blood depends on the lipid content (Yeh & Peterson, 1965), lipid was measured by Folch's technique (Folch, Lee & Sloane-Stanley, 1957). The lipid values (mg/100 g testis) were 4.95 (pool of 4 testes) for young foxes and 4.45 ± 1.33 ($n = 20$) and 7.95 ± 4.48 ($n = 15$) for adult foxes in and out the breeding season respectively: the differences were not significant. The partition coefficient of xenon (λ) has not been determined for the fox testis. The value given by Fritjofsson, Persson & Pettersson (1969) ($\lambda = 0.82$) for the human testis, which is structurally very similar to the fox testis (Joffre, 1976b), was used to determine the TCBF of foxes by the formula of Kety (1951). The specific gravity of the testis (ρ) was determined on 65 foxes and no changes were found throughout the reproductive life (1.063 ± 0.005 g/ml).

Plasma testosterone levels were determined by the radioimmunoassay method of Leymarie, Strauss & Scholler (1974), validated for the fox by Joffre, M. & Joffre, J. (1975), in blood samples collected from the radial vein, just after the determination of TCBF. The antibody was raised in a rabbit against testosterone-3-(O-carboxy)-methyloxine-BSA and the working range of the assay was 5–140 pg. The inter- and intra-assay variations were 3.2% and 7.3% respectively; each sample being estimated in duplicate. The spermatogenic activity of the testis was estimated from its weight (Joffre, 1976a) and histology at the time of autopsy.

Statistical significance was determined by using the Student's *t* test. Correlation techniques were used to evaluate the relations between TCBF and testicular weight or plasma testosterone throughout the year.

Results

The variations of TCBF, measured at monthly intervals, in young and adult foxes are shown in Table 1.

Table 1. Seasonal changes of mean $\pm$ S.E.M. testicular capillary blood flow ($\text{ml min}^{-1}\text{g}^{-1}$) of young and adult anaesthetized wild red foxes (no. of measurements in parentheses)

	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June
Young foxes	0.34 $\pm$ 0.04 (9)	0.25 $\pm$ 0.03 (26)	0.31 $\pm$ 0.03 (25)	0.38 $\pm$ 0.03 (51)	0.42 $\pm$ 0.03 (44)	0.46 $\pm$ 0.04 (29)	0.62 $\pm$ 0.04 (38)	0.50 $\pm$ 0.03 (56)	0.27 $\pm$ 0.02 (23)	0.22 $\pm$ 0.02 (69)	0.12 $\pm$ 0.01 (37)	0.10 $\pm$ 0.01 (85)
Adult foxes	0.18 $\pm$ 0.02 (30)	0.14 $\pm$ 0.02 (23)	0.32 $\pm$ 0.02 (29)	0.31 $\pm$ 0.02 (29)	0.34 $\pm$ 0.03 (19)	0.43 $\pm$ 0.04 (19)	0.65 $\pm$ 0.03 (72)					

TCBF and spermatogenic activity

As shown in Text-fig. 1, TCBF and testicular weight are closely correlated during the reproductive life of young and adult foxes ($r = 0.67$; $P < 0.01$), except during the prepubertal period of the young and, in September, at the beginning of the period of testicular regrowth of the adult when TCBF increases before testicular weight.

Text-fig. 1. Seasonal changes (monthly means) of testicular capillary blood flow (■), testicular weight (□) and plasma testosterone levels (▲) in young (A) and adult (B) red foxes. The figures along the horizontal axis represent the number of animals studied in each month. See Table 1 for numbers involved in TCBF measurements. Arrow indicates time of birth.

It was not possible to determine the TCBF of young foxes until June because of the difficulty of locating the testis under the scrotum and its small size for injecting xenon. From July until October, while the testis was slowly growing and gonocytes were multiplying, TCBF did not vary significantly (0.29 ± 0.03 ml min⁻¹ g⁻¹, $n = 60$). In October, when TCBF and testicular weight began to increase, spermatogonia, primary spermatocytes, spermatids and spermatozoa appeared in the seminiferous tubules. In January, TCBF reached a maximum value and the foxes were then pubescent, testicular weight also being maximal (5358 ± 207 mg, $n = 16$) and numerous spermatozoa were present in the seminiferous tubules. From February until May, TCBF and testicular weight progressively decreased and, at the beginning of this period, spermatogenesis ceased, leaving degenerate cells in the tubule lumina which were evacuated through the epididymis. In March, only a few primary spermatocytes were observed in the seminiferous tubules. From May until September, the resting period, testicular weight was low (1122 ± 54 mg, $n = 24$), the TCBF was very much lower than the values in immature foxes (0.12 ± 0.01 ml min⁻¹ g⁻¹, $n = 125$, $P < 0.01$) and there was little mitotic activity in the seminiferous tubules which contained few primary spermatocytes (zygotene), spermatogonia and, sometimes, only Sertoli cells. Then, in September, sometimes in August, TCBF rapidly increased ($P < 0.01$) but there was no concomitant increase of testicular weight. At this time, TCBF values were very similar to those of prepubertal foxes at the same period of the year. Many primary spermatocytes were present in the seminiferous tubules of 4 foxes killed at this time and spermatogenesis proceeded actively. In January, during the mating period, TCBF reached its maximum value and was associated with the highest testicular weight (6759 ± 350 mg, $n = 19$) and full spermatogenesis. From October to January, during the prepubertal period and the period of testicular regrowth of the adult, fluctuations of TCBF tended to occur simultaneously in both testes of an individual but these rarely coincided in different foxes. Temporary changes of TCBF were observed during the resting period of the adult, but they were never observed on consecutive days.

TCBF and androgenic activity

TCBF and plasma testosterone levels were closely correlated at all times ($r = 0.61$, $P < 0.01$), except during the period of testicular regrowth of the adult when the TCBF increased before the

testosterone level altered. High TCBF values were normally associated with high testosterone levels and the presence of spermatozoa in the seminiferous tubules but, during the resting period, high levels of testosterone were sometimes associated with a low TCBF and *vice versa*.

Discussion

The radioactive inert gas clearance technique was considered to give an exact measurement of TCBF (Setchell, 1970), but Einer-Jensen (1974), by using a miniature Geiger-Muller tube inserted into the testis, has shown that the xenon recirculates and that only the first fast component of the clearance curve (range 1 to 5 min) gives a suitable value of TCBF, confirming our previous results (Joffre, M. & Joffre, J., 1971). In rats (Joffre, M. & Joffre, J., 1971) and rams (Courot & Joffre, 1976), this technique gives blood flow values similar to those of other techniques (Setchell, 1970). However, this is the only method available when repeated measurements of TCBF have to be made in the same animal throughout the year, as in the present study.

TCBF is very high during the mating period of the adult when numerous spermatozoa are present in the seminiferous tubules and there are high levels of testosterone circulating in the peripheral plasma. All the present results corroborated those previously published (Joffre, J. & Joffre, M., 1973), but the values for TCBF are higher than those determined in other mammals (Setchell, 1970), including ferret, dormouse (Joffre, J. & Joffre, M., 1973) and European badger (M. Joffre, unpublished data). This may be related to the short duration of the breeding period and the consequent need for a high nutrient blood flow as well as the profuse vascular system in this species (Joffre, M. & Kormano, 1975). At puberty, TCBF is lower than that previously reported (Joffre, M., 1973a), because all clearance curves were re-investigated and some were excluded because of the possibility of the testis being hyperaemic. The maximal values in the pubescent and adult fox were similar despite the differences of testicular weight and testosterone levels in such animals. The low TCBF value in immature foxes is higher than that determined for lambs (Courot & Joffre, 1976) or young rats (J. Joffre, unpublished data). During the resting period of the adult, TCBF is minimal and is $2\frac{1}{2}$ times lower than TCBF in immature foxes, although the testicular weight is twice as great.

In mammals, TCBF is under nervous control (Setchell, 1970), but it seems unlikely that seasonal changes of TCBF are regulated by catecholamines. Nevertheless, the simultaneous temporary changes observed in both testes of young and adult foxes from October to January might be induced by catecholamines released during stress induced by handling of the animals. In the ram, a decrease of body weight after a long period of undernutrition lowers the total blood flow per unit of testicular weight (Setchell, Waites & Lindner, 1965). There are no seasonal changes in the body weight of the adult fox and, in the young, the increase of TCBF occurs while the body weight approaches the adult value. Changes of TCBF have been observed during the breeding cycle of other mammals (Joffre, J. & Joffre, M., 1973; Courot & Joffre, 1976), but changes of TCBF during the prepubertal period have not been found for rabbits (Larson & Foote, 1974) or lambs (Courot & Joffre, 1976).

The regulation of capillary blood flow is under metabolic control in numerous vascular beds (Haddy & Scott, 1975). It is generally agreed that the TCBF varies only slightly in the testis while testicular metabolism changes (Waites & Setchell, 1969a, b; Setchell, 1970). Nevertheless, TCBF does appear to be correlated with the metabolism of the gonad in the fox: in September, the increase of TCBF occurs with the onset of spermatogenesis, without growth of testicular size and despite the low testosterone level, suggesting that TCBF is primarily under a pituitary hormonal and (or) metabolic control at the microvascular level.

In this study, TCBF was similar in the pubescent and adult foxes, in spite of the different testosterone levels, and in the adult TCBF increased in the autumn before the appearance of high testosterone levels. These results suggest that TCBF is independent of the androgenic activity of the testes, a supposition corroborated by the fact that injections of testosterone into adult foxes during the resting period (Joffre, M., 1977a) and of cyproterone acetate into rats (Joffre, M., 1973b) do not change the TCBF.

In young and adult foxes, the changes of TCBF occur without any modification of the capillary

network (Joffre, M., 1976b), but during the period of testicular regression in the adult and, conversely, during the growth of testis, changes of TCBF are closely associated with the appearance and the disappearance of spiral arteries in the testicular parenchyma (Joffre, M. & Kormano, 1975). Blood pressure is known to decrease along spiral arteries (Waites & Moule, 1960; Moll & Kunzel, 1971, 1973), and it therefore seems possible that the spiral arteries are the cause of the reductions in TCBF in foxes. Moreover, during testicular regression, the capsule and septa of the testis lose weight and total collagen (Joffre, M., 1977b). Except for the capillaries, most of the testicular vessels are closely associated with the capsule and septa and these appear to be responsible for the cyclic changes of testicular vessels and, consequently, of TCBF. In the rat, in which the testis is organized without septa, hypophysectomy or cryptorchidism involve no important change of TCBF (Glover, 1965; Csernay, Lazlo & Kovacs, 1968; Setchell, Voglmayr & Waites, 1969) and no modification of the testicular vasculature (Harrison, 1949; Kormano, 1969).

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