Xenografting of adult mammalian testis tissue

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Abstract

Xenografting of testis tissue from immature males from several mammalian species to immunodeficient mouse hosts results in production of fertilization-competent sperm. However, the efficiency of testis tissue xenografting from adult donors has not been critically evaluated. Testis tissue xenografting from sexually mature animals could provide an option to preserve the genetic material from valuable males when semen for cryopreservation cannot be collected. To assess the potential use of this technique for adult individuals, testes from adult animals of six species (pig, goat, cattle, donkey, horse and rhesus monkey) were ectopically grafted to host mice. Grafts were recovered and analyzed at three time points: less than 12 weeks, between 12 and 24 weeks and more than 24 weeks after grafting. Histological analysis of the grafts revealed effects of species and donor tissue maturity: all grafts from species with greater daily sperm production (pig and goat) were found to have degenerated tubules or grafts were completely degenerated. None of the xenografts from mature adult bull and monkeys contained differentiated spermatogenic cells when examined more than 12 weeks post-grafting but tubules with Sertoli cells only remained. In grafts from a young adult bull, Sertoli cells persisted much longer than with the mature adult grafts. In grafts from a young adult horse, spermatogenesis proceeded to meiosis. In grafts from a young adult donkey and monkey, however, complete spermatogenesis was found in the grafts. These results show that testis tissue grafts from mature adult donors did not support germ cell differentiation but seminiferous tubules with Sertoli cells only survived in some species. The timing and progression of tubular degeneration after grafting of adult testis tissue appear to be related to the intensity of spermatogenesis at the time of grafting. Testis tissue from sub-adult donors survives better as xenograft than tissue from mature adult donors, and complete spermatogenesis can occur albeit with species-specific differences.

Keywords

Testis; adult; graft

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1. Introduction

Testis tissue grafting presents an option for preservation of genetic material from individual males when sperm recovery is not possible. Grafting of testis tissue from sexually immature males to immunodeficient mice results in germ cell differentiation and production of sperm from different mammalian species like pigs and goats (Honaramooz et al., 2002), hamsters (Schlatt et al., 2002), rabbits (Shinohara et al., 2002), bulls (Oatley et al., 2004), monkeys (Honaramooz et al., 2004), cats (Snedaker et al., 2004) and horses (Rathi et al., 2006). However, the efficiency of testis tissue xenografting from adult donors has not been critically evaluated (Pukazhenthi et al., 2006). Spermatogenesis was arrested at meiosis in grafts from mature horse (Rathi et al., 2006) and photoregressed hamster testis (Schlatt et al., 2002) while no germ cell differentiation occurred in xenografts of adult human testis tissue (Schlatt et al., 2006; Geens et al., 2006) indicating species specific differences in progression of germ cell differentiation in testis grafts from sexually mature donors. Complete spermatogenesis with the presence of elongated spermatids in the xenografted tissue has not been reported when donor testis tissue was sexually mature and contained post-meiotic germ cells at the time of grafting in any species and most of the grafts regressed or contained degenerated tubules. It was suggested that mature testis tissue is not able to survive after xenografting (Rathi et al., 2006) perhaps due to a greater sensitivity to ischemia compared with immature tissue or potentially because it is less effective in inducing angiogenesis (Schlatt et al., 2002).

Xenografting of testis tissue has its greatest utility in preservation of genetic material from immature individuals where cryopreservation of sperm is not an option. However, in cases where spermatozoa cannot be obtained for immediate preservation, in species with seasonal reproductive patterns where spermatogenesis is suppressed outside the breeding season (Blottner et al., 1995) or if an individual is rendered azoospermic by disease or cytotoxic therapy for treatment of cancer, application of xenografting to adult donor tissue would present a novel alternative for fertility preservation (Orwig and Schlatt, 2005). In addition, testis grafts provide an efficient system to study the effects of drugs or toxins on mammalian spermatogenesis without the need for extensive experimentation in the donor species (Jahnukainen et al., 2006) or to study the pathogenesis underlying disturbances of spermatogenesis (Turner et al., 2006). It would, therefore, be desirable to extend the utility of testis tissue xenografting to donor tissue from adult donors. The objective of the present study was to investigate survival and germ cell differentiation of testis xenografts from sexually mature donors of different species.

2. Materials and methods

2.1. Animals and tissues

Testes of 13 donor animals from six species were included in this study: two pigs (8 and 11 months old), three goats (18 months and two 4 years old), two bulls (7 months and 3 years old), one donkey (13 months old), one horse (2 years old) and four rhesus monkeys (3, 6, 11 and 12 years old). The xenografting procedure was conducted as described previously (Honaramooz et al., 2002). Briefly, immunodeficient, male (6-8 wk old) NCR nu/nu (n = 18) or SCID (n = 29) mice were castrated and during the same anesthesia eight small pieces of donor testis were ectopically grafted under the back skin of each mouse (2-7 mice/donor). Care and use of research animals were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. Grafting was performed between February 2002 and December 2005 as part of other experiments that did not include analysis of grafts from adult donors, therefore, the number of mice and the exact time points for removing the grafts were different for different donors. To compare the development of the grafts among species, time points of graft recovery were grouped into three categories: less than 12 weeks, between 12 and 24 weeks and more than 24 weeks (between 24 and 38 weeks).

Donor tissue and grafts were fixed in Bouin's solution followed by three changes in 70% ethanol and processed for histological analysis. Seminal vesicle weight as an indicator of bioactive testosterone production by the grafted tissue and graft size were recorded. Grafts were classified as completely degenerated when no tubules were found. When seminiferous tubules were observed, all of these were analyzed and classified either as degenerated tubules when the content was amorphous (hyalinized tubules) or, when spermatogenesis was present, according to the most advanced type of germ cell found. The percentage of recovered grafts was calculated for all mice where grafts were found, and it was recorded when no grafts were recovered.

2.2. Statistical analysis

Fisher's exact test was performed (Siegel and Castellan, 1988) to compare the proportion of successful/unsuccessful grafts and mice between groups. Grafts were classified as success (or healthy grafts, when they contained some tubules that were not degenerated) or failure (degenerated grafts or grafts where all tubules were degenerated). Mice were classified as success (when they carried grafts where some tubules were not degenerated) or failure (when all the grafts present were degenerated grafts or grafts contained only degenerated tubules). Differences associated with $P<0.05$ were considered significant.

3. Results

3.1. Pig

In the donor testes more than 85% of the seminiferous tubules contained elongated spermatids (Table 1, Figure 1A). Only $31.3\% \pm 12.5$ (n = 4 mice) of the grafts were recovered before 24 weeks post-grafting and all of these were completely degenerated (Figure 1B). After 24 weeks, 25% of the grafts were recovered in one mouse but no visible tubules were observed (Table 2A).

3.2. Goat

The percentage of tubules with elongated spermatids as the most advanced germ cell type present was greater than 75% in all three donor goats (Table 1, Figure 1C and 1E). From the 18 month old goat, all the grafts recovered before 12 weeks (75%, n = 1 mouse) and after 24 weeks ($43.8\% \pm 8.8$, n = 2 mice) were completely degenerated and no visible tubules were found (Figure 1D). From the two 4 year old goats, all the grafts recovered before 24 weeks ($92.5\% \pm 11.2$, n = 5 mice) contained tubules but all of the tubules analyzed (n = 323) were degenerated (Table 2A, Figure 1F).

3.3. Bull

In the donor tissue from the 3 year old bull, 72.8% of tubules contained elongated spermatids (Table 1, Figure 1G). At less than 12 weeks the grafts contained Sertoli cells and degenerated tubules (Table 2A, Figure 1H), and the percentage of degenerated tubules increased from 65.2% at 2 weeks to 83.3% at 4 weeks and 95.2% at 8 weeks post-grafting. After 12 weeks the grafts contained degenerated tubules only (Figure 3). Overall, $78.1\% \pm 21.3$ (n = 4 mice) of grafts were recovered and 128 tubules were analyzed. The testis tissue from the 7 month old bull contained 9.2 % of seminiferous tubules with elongated spermatids as the most advanced germ cell type present (Table 1, Figure 2A). More than 24 weeks post-grafting $62.5\% \pm 17.7$ (n = 2 mice) of grafts were recovered and showed $84.6\% \pm 1.4$ of tubules (n = 184) with Sertoli cells (Table 2B, Figure 2B).

3.4. Rhesus monkey

The four donors presented complete spermatogenesis in more than 66% of seminiferous tubules (Table 1, Figure 1I, 1K and 2C). The average graft recovery from the two older monkeys less

than 12 weeks post-grafting was 62.5% ± 17.7 (n = 2 mice) and grafts contained tubules (n = 70) with 38.9% ± 43.5 having Sertoli cells only and 61.1% ± 43.5 with degenerated tubules (Figure 1J). Graft recovery was less 12.5% (n = 1 mouse) between 12 and 24 weeks and 25% ± 17.7 (n = 2 mice) more than 24 weeks post-grafting with no visible tubules found in any of the grafts. From the 6 year old monkey, 81.3% ± 8.8 (n = 2 mice) of grafts were recovered less than 12 weeks post-grafting; these contained seminiferous tubules (n = 165) with 32.9% ± 39.8 with Sertoli cells only, 0.3% ± 0.5 with pachytene spermatocytes and 66.7% ± 40.3 were degenerated (Figure 1L). After 24 weeks post-grafting, graft recovery was 75% (n = 1 mouse) with only 20 seminiferous tubules found containing 45% degenerated tubules and 55% with Sertoli cells only. Between 12 and 24 weeks, 85.0% ± 16.3 (n = 5 mice) of the grafts were recovered from the youngest donor (3 years old) with 0.1% ± 0.2 of tubules showing elongated spermatids (n = 1848 tubules). After 24 weeks post-grafting, 37.5% of the grafts were recovered (n = 1 mouse) containing 1.1% with elongated spermatids (n = 184 tubules) (Table 2B, Figure 2D). Grafts from the youngest monkey had greater development than grafts from the three older animals (p = 0.002, n = 65 grafts). Figure 4 illustrates the percentage of tubules containing the most advanced germ cell stage present in grafted tissues.

Grafts from pig and goat testes degenerated in a few days after grafting. In these two species, spermatogenesis is very efficient as evidenced by a greater daily sperm output. In contrast, in grafts from monkey testes not all the tubules were degenerated even at the last time point analyzed and tubules containing only Sertoli cells were found more than 24 weeks postgrafting. Accordingly, the proportions of unsuccessful grafts (p < 0.001, n = 90) and mice (p = 0.0017, n = 23) were greater for testes from pigs and goats than for those from monkeys.

3.5. Donkey and horse

The donkey donor testis tissue contained 33.2% with elongated spermatids as the most advanced germ cell type (Table 1, Figure 2E). Between 12 and 24 weeks post grafting, the percentage of recovered grafts was 37.5% ± 0.0 (n = 2 mice) with 97.4% ± 3.7 of them containing Sertoli cells only and 2.6% ± 3.7 containing spermatocytes (n = 156 tubules). In another mouse, 100% of the grafts were recovered with 999 seminiferous tubules and complete spermatogenesis was found in 0.8% of tubules (Table 2B, Figure 2F), 6.0% of tubules contained round spermatids, 45.7% pachytene spermatocytes and 47.5% contained only Sertoli cells. The testes of the 2 year old horse did not provide indications of complete spermatogenesis but round spermatids were observed in 0.3% of the tubules (Table 1, Figure 2G). Between 12-24 weeks post-grafting, 45.8% ± 7.2 (n = 3 mice) of the grafts were recovered and 1.7% ± 1.8 of seminiferous tubules (n = 354) contained pachytene spermatocytes (Table 2B, Figure 2H). After 24 weeks post-grafting, the percentage of recovered grafts was 37.5% ± 12.5 (n = 3 mice). All the grafts from one mouse were completely degenerated, another contained 100% degenerated tubules and the other 96.7% seminiferous tubules cotaining Sertoli cells only and 3.3% with degenerated tubules (n = 293).

3.6. Graft size and seminal vesicle weight

The average size of the recovered grafts was 2.0 ± 2.3 mm^3 (mean ± SD, n = 182 grafts). The weights of the seminal vesicles (SV) were categorized as above or below 50 mg: in mice with SV smaller than 50 mg, the average weight was 15.1 ± 6.1 (range = 6 to 30 mg; n = 33) and in the mice with SV larger than 50 mg it was 103.5 ± 52.2 (range = 60 to 180 mg; n = 8). When grafts were recovered at a very early time point (before 5 weeks after castration and grafting) the SV weight was still high (80 mg; n = 2). Between 12 and 24 weeks the mice with donkey and horse testis grafts had greater SV weights (128 and 80 mg, respectively). For the last time point analyzed, the SV weight from the mice with 7 month old bull grafts was 180 mg (n = 2) and 60 and 80 mg from the mice with horse grafts.

4. Discussion

The donor testis tissue used in the present study can be classified into two groups based on testicular histology prior to grafting: (1) sexually mature adults with more than 70% of seminiferous tubules containing elongated spermatids and all tubules with differentiated germ cells and (2) sub-adults or young adults with less than 70% of tubules with or without elongated spermatids present and some tubules without differentiated germ cells.

None of the xenografts from mature adult donors contained seminiferous tubules with differentiated cells more than 12 weeks after grafting. In addition, it appeared that the timing and progression of degeneration depended on the spatial organisation of the germ cells in the seminiferous epithelium at the time of grafting and on the intensity of spermatogenesis that, in turn, is species dependent. The species represented in this work can be divided into three types depending on their efficiency of sperm production. Pigs and goats have a greater daily sperm output per gram testis tissue, about 30×10^6 sperm in the boar and 27×10^6 sperm in the ram (Johnson et al., 1970). If the donor tissue shows evidence of efficient spermatogenesis as observed in the pigs (Figure 1A) and the 18 month old goat (Figure 1C), the degeneration of the grafted tissue is more rapid and occurs within 5 weeks post-grafting with no tubular structures remaining (Figure 1B and 1D). In donor tissue with less intense spermatogenesis at the time of grafting, as observed in the two 4 year old goats (Figure 1E), the degeneration is slower and degenerated tubules can be observed at 20 weeks post-grafting; however, no somatic or germ cells remain in the tubules (Figure 1F).

Daily sperm production, as an indication of spermatogenic efficiency, is less in bulls (18×10^6 sperm/gram testis tissue; Johnson et al., 1970) than in pigs or goats. Accordingly, the degeneration of the tissue after grafting was slower than in tissue from goats and pigs and tubules with Sertoli cells were present until 8 weeks post-grafting (Figure 1H). However, with increasing time after grafting, all the tubules degenerated as with pigs and goats.

In rhesus monkeys, average daily sperm production per gram testis tissue is 23×10^6 (Johnson et al., 1970), intermediate between pigs or goats and bulls. Before 4 weeks post-grafting, seminiferous tubules with Sertoli cells were observed (Figure 1J). However, at later time points tubules degenerated and by 12 and 24 weeks post-grafting the grafts were completely degenerated. One sample analyzed from the 6 year old monkey donor contained pachytene spermatocytes in a few tubules at an early analysis time point. However, it is likely that these cells remained from the donor tissue and were not generated in the graft. In addition, the seminal vesicle weight was less, indicating absence of testosterone production by the graft. Interestingly, spermatogenesis at the time of grafting in this donor appeared less efficient histologically (Figure 1K) than in the other two mature adults (Figure 1I) and the degeneration of the tubules was slower and tubules containing only Sertoli cells were still present more than 24 weeks post-grafting (Figure 1L). This finding is in agreement with earlier reports that human testis tissue from individuals with suppressed spermatogenesis had enhanced survival as xenografts than that from donors with complete spermatogenesis at the time of grafting (Schlatt et al., 2006).

Four of the 12 donors used in the present study were sub-adult animals. The development of the grafts from young adult tissue was different from the grafted-mature-adult tissue. In the sample from the younger bull, germ cell differentiation did not occur, but Sertoli cells persisted much longer than with mature adult grafts (Figure 2B). Similarly, in xenografts of the 2 year old horse testis, few pachytene spermatocytes were found between 12 and 24 weeks post-grafting (Figure 2H) but no further development occurred. In contrast, in the young monkey and donkey, although the donor tissue was histologically more developed, complete spermatogenesis occurred in the grafts (Figure 2D and 2F) and the percentage of elongated

spermatids increased slightly with time. Therefore, it appears that testis tissue from sub-adult donors has enhanced survival as a xenograft than tissue from mature adult donors, and in some cases complete spermatogenesis can occur. However, while the histological appearance of the donor tissue at the time of grafting appears to be related to tissue survival and germ cell differentiation after grafting, species-specific differences exist.

The reasons for poor survival of adult testis tissue xenografts are so far unknown. It was proposed that Sertoli cells lose the capacity of division when sexual maturity is reached (O'Donnell et al., 2006) and that this precludes development of the grafts. Lack of Sertoli cell proliferation could explain that grafts from adult donors did not grow as much as those from immature donors and that spermatogenesis arrested at a given stage. However, lack of Sertoli cell proliferation is unlikely to be the cause for the complete degeneration of the tubules and death of all cell types that occurs in testis grafts from adults with greater spermatogenesis at the time of grafting. Moreover, proliferation of Sertoli cells in adult tissue of seasonal breeders has been reported (Russell, 1993), particularly in stallions (Johnson and Thompson, 1983) and the photosensitive Djungarian hamster (Meachem et al., 2005), but previous work with these species indicated no success in xenografting of adult tissue (Schlatt et al., 2002; Rathi et al., 2006).

After xenografting of testis tissue with differentiated germ cells present at the time of grafting, most of the advanced germ cells present in the tissue died and spermatogenesis was reinitiated from the remaining spermatogonia (Rathi et al., 2006). The metabolism of round spermatids in rats is exclusively oxygen dependent (Bajpai et al., 1998), therefore, it is possible that transient ischemia during and immediately after grafting induces the death of round spermatids but spermatogonia and some spermatocytes could survive. However, the death of all types of cells and the degeneration of the graft can not be explained by either of these theories. While the different types of germ cells may have different sensitivity to hypoxia during spermatogenesis, testis tissue as a whole is metabolically very active making it potentially more sensitive to transient anoxia caused by the grafting procedure. Grafts of adult tissue appear to be well vascularized similar to those of immature testis (data not shown) but adult tissue could be more sensitive to ischemia (Schlatt et al., 2002). Testis tissue with lesser spermatogenesis at the time of grafting may be less sensitive to hypoxia and, therefore, have enhanced survival as observed in the present study. In support of this hypothesis, testis tissue was observed in the present study where some tubules did not contain differentiated germ cells had enhanced survival as xenografts which could be due to a lesser rate of metabolism at the time of grafting. However, the observed species-specific differences cannot be explained by this hypothesis and require further investigation. Recently, it was reported that pre-treatment of testis grafts with vascular endothelial growth factor improved germ cell differentiation in xenografts of immature bovine testis tissue (Schmidt et al., 2006). Whether the implied effect on improved vascularization would be beneficial for the survival of adult testis tissue remains to be investigated.

Mice that had grafts from the mature adult donors generally had small seminal vesicles (less than 30 mg) indicating less testosterone production but some mice with grafts from the young adult donors had seminal vesicles that weighed more than 50 mg. It appears, therefore, that Leydig cells in the mature adult tissue grafts are not able to produce physiologic amounts of testosterone or that the tissue degenerates so rapidly that production of testosterone does not occur. The majority of mice with grafts that showed germ cell differentiation (donkey and horse) had large seminal vesicles but not all the grafts from mice with greater SV weights contained differentiated germ cells (e.g., in grafts of testis tissue from the 7 month old bull). Therefore, production of testosterone is needed but not sufficient for germ cell differentiation in testis tissue grafts.

5. Conclusions

Testis tissue from mature adult donors did not result in germ cell differentiation when grafted to mice but seminiferous tubules with Sertoli cells only survived up to 12 or 24 weeks in grafts from donor species with moderate to minimal sperm production. The histological appearance of the donor tissue at the time of grafting appears to be related to the progression of degeneration of grafts from mature adult donors. When the tissue shows evidence of efficient spermatogenesis, regardless of species, degeneration occurs faster. However, when the donor tissue is not completely mature, complete spermatogenesis can be found in grafts, albeit with species-specific differences. Based on the observations in the present study, xenografting of testis tissue from adult donors appears more promising for the preservation of germ cells in horses and primates, and in individuals that do not have highly efficient spermatogenesis. Therefore, histological appearance of spermatogenic tissue at the time of grafting can serve to predict xenograft survival and potential for germ cell differentiation.

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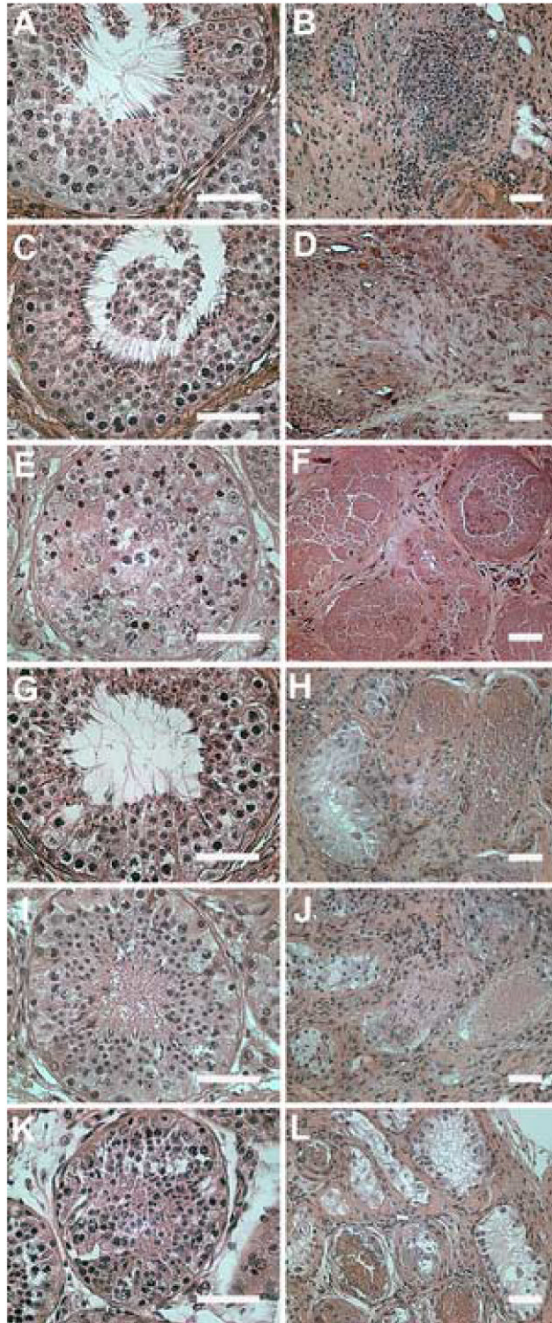
Fig. 1.
Histological appearance of mature adult donor testis tissue (left) and testis tissue xenografts less than 12 weeks post-grafting (right). A, B: Pig (8 months old), C, D: Goat (18 months old), E, F: Goat (4 years old), G, H: Bull (3 years old), I, J: Rhesus monkey (12 years old), and K, L: Rhesus monkey (6 years old). Scale bars = 50 μm.
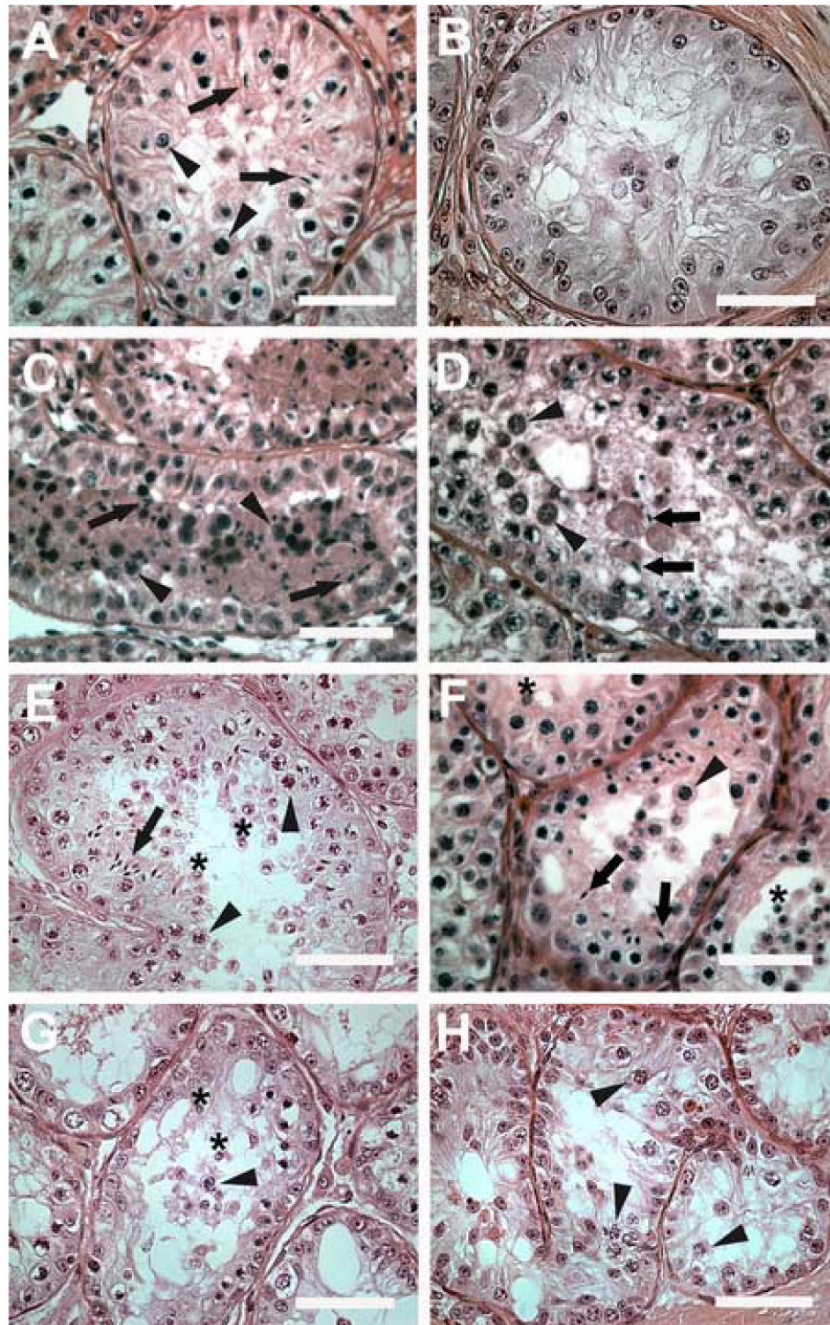
Fig. 2.
Histological appearance of sub-adult donor tissue (left) and grafts (right). A: Bull 7 months old, B: graft >24 weeks post-grafting; C: Monkey 3 years old, D: graft >24 weeks post-grafting; E: Donkey 13 months old, F: graft 12-24 weeks post-grafting and G: Horse 2 years old, H: graft 12-24 weeks post-grafting. Elongated spermatids are indicated with arrows, pachytene spermatocytes with arrow heads and round spermatids with asterisks. Scale bars = 50 μm.
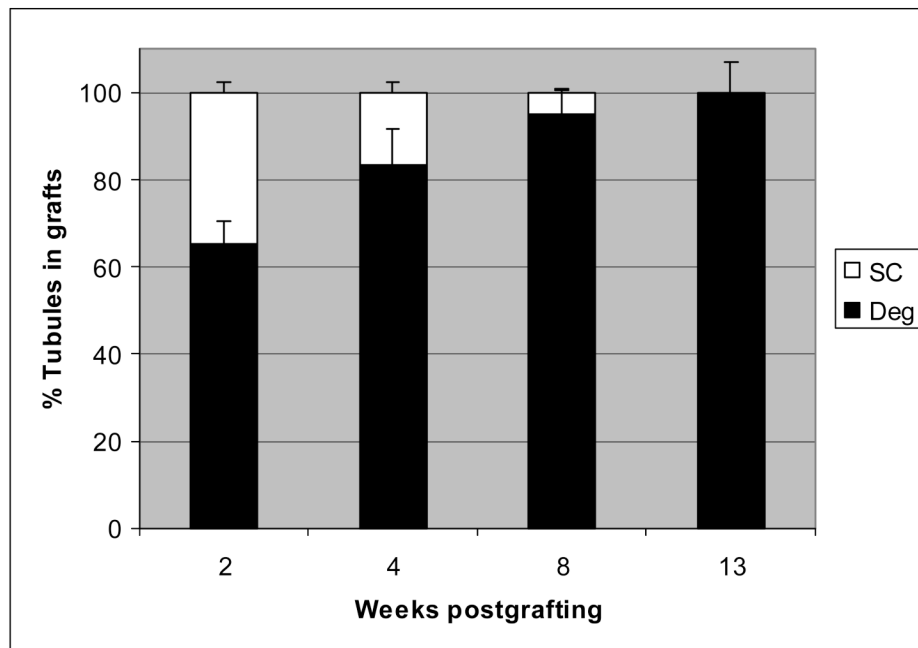
Fig. 3.
Percentage of degenerated and 'Sertoli cells only' tubules in xenografts from mature adult bovine testes at different weeks after grafting. Deg: degenerated, SC: Sertoli cells only. Mean ± SEM, n = 23 grafts.
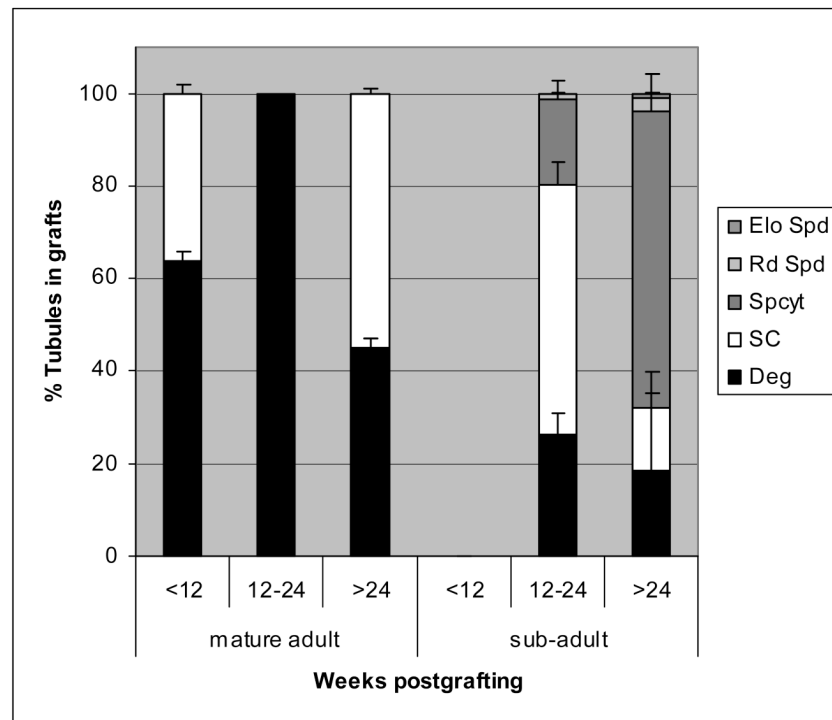
Fig. 4.
Percentage of seminiferous tubules with the most advanced germ cell type in mature adult and sub-adult monkeys by time point after grafting. Deg: degenerated, SC: Sertoli cells only, Spcyt: pachytene spermatocytes, Rd Spd: round spermatids and Elo Spd: elongated spermatids. Mean ± SEM, n = 57 grafts.

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Table 1

Percentage of seminiferous tubules with the most advanced germ cell stage present in the donor tissue

| Species	Age of donor	No. of tubules analyzed	% Tubules with most advanced cell type			
			SC	Spcyt	Rd Spd	Elo Spd
Pig	8 months	669	0.0	2.1	9.7	88.2
	11 months	287	0.0	2.8	11.1	86.1
Goat	18 months	619	0.0	0.9	23.9	75.2
	4 years	233	0.0	2.2	10.7	87.1
	4 years	174	0.0	1.7	21.3	77.0
Bull	7 months	809	11.9	49.1	29.9	9.2
	3 years	184	0.0	8.2	19.0	72.8
Monkey	3 years	534	4.9	13.3	15.0	66.9
	6 years	730	0.0	3.0	20.8	76.2
	11 years	807	0.0	1.1	12.6	86.3
	12 years	371	0.0	4.9	17.5	77.6
Donkey	13 months	742	2.7	32.8	31.4	33.2
Horse	2 years	620	23.2	76.5	0.3	0.0

SC: Sertoli cells only, Spcyt: spermatocytes, Rd Spd: round spermatids and Elo Spd: elongated spermatids.

Table 2

Experimental design and analysis of tissue grafts by species and after grafting

A) Grafts from mature adult donors

Species (n)	Time point (months)	Number of mice[1]	Number of grafts[2]	Degenerated grafts[3]	% tubules in non degenerated grafts		with cell type		
					Degenerated	SC	Spcyt	Rd Spd	Elo Spd
Pig (2)	<12	3/3	7/24	100(7)	N/A	N/A	N/A	N/A	N/A
	12-24	1/1	3/8	100(3)	N/A	N/A	N/A	N/A	N/A
	>24	1/4	2/32	100(2)	N/A	N/A	N/A	N/A	N/A
Goat (3)	<12	2/2	13/16	46.2(6)	100	N/A	N/A	N/A	N/A
	12-24	4/4	30/32	N/A	100	N/A	N/A	N/A	N/A
	>24	2/2	7/16	100(7)	N/A	N/A	N/A	N/A	N/A
Cattle (1)	<12	3/3	18/24	N/A	81.2	18.8	N/A	N/A	N/A
	12-24	1/1	7/8	N/A	100	N/A	N/A	N/A	N/A
	>24	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Monkey (3)	<12	4/4	23/32	N/A	63.9	35.9	0.2	N/A	N/A
	12-24	1/1	1/8	100(1)	N/A	N/A	N/A	N/A	N/A
	>24	3/3	10/24	50(5)	45	55	N/A	N/A	N/A

B) Grafts from sub-adult donors

Species (n)	Time point (months)	Number of mice[1]	Number of grafts[2]	Degenerated grafts[3]	Degenerated	SC	Spcyt	Rd Spd	Elo Spd
Cattle (1)	<12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	12-24	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	>24	2/2	10/16	N/A	15.4	84.6	N/A	N/A	N/A
Monkey (1)	<12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	12-24	5/5	34/40	N/A	26.4	53.8	18.6	1.1	0.1
	>24	1/2	3/16	N/A	18.5	13.6	64.1	2.7	1.1
Donkey (1)	<12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	12-24	3/5	14/40	N/A	N/A	80.8	17.0	2	0.3
	>24	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Horse (1)	<12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	12-24	3/3	11/24	N/A	26.9	71.4	1.7	N/A	N/A
	>24	3/3	9/24	22.2(2)	51.6	48.4	N/A	N/A	N/A

[1] Mice with grafts recovered /total grafted mice.

[2] Grafts recovered /transplanted grafts.

[3] Percentage and (number) of degenerated grafts (with no tubules found).

SC: Sertoli cells only, Spcyt: pachytene spermatocytes, Rd Spd: round spermatids and Elo Spd: elongated spermatids. N/A: not applicable.