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·*Original Article*·

Development of neonatal mouse and fetal human testicular tissue as ectopic grafts in immunodeficient mice

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Abstract

Aim: To investigate the stepwise development and germ cell gene expression in allografted neonatal mouse testes and the differentiation of immature human testicular cells in xenografted human testes. **Methods:** Immunodeficient nude mice were used as hosts for allografting of neonatal mouse testes and xenografting of human fetal testicular tissues. Stepwise development and stage-specific gene expression of germ cells in allografts were systematically evaluated and parallel compared with those in intact mice by periodically monitoring the graft status with measurement of graft weight, histological analysis and determination of five stage-specific genes. Human testicular tissues from 20 and 26 weeks fetuses were used for the xenografting study. Histological analysis of xenografts was performed 116 and 135 d after the grafting procedure. **Results:** In the allografting study, progressive increase in tissue volume and weight as well as in tubule diameter in grafts was observed; the appearance time of various germ cells in seminiferous tubules, including spermatogonia, spermatocytes, round and elongate spermatids and sperm, was comparable with that in intact donors; the initiation of gene transcription in grafts showed a similar trend as in normal mice. Graft weight ceased to increase after 7–8 weeks and degradation of grafts was observed after 5 weeks with progressive damage to seminiferous epithelium. In the xenografting study using immature human testicular tissues, graft survival and development was indicated by increasing graft weight, Sertoli cells differentiation into advanced stage, germ cells migration and location to the basal lamina and formation of a niche-like structure. **Conclusion:** The developmental course and gene expression pattern of germ cells in allografts were similar to those in intact mice. The best time point for retrieval of mouse sperm from grafts was 5–7 weeks after grafting procedure. An accelerated development of immature human testicular cells could be achieved by ectopic xenografting of human testes. *(Asian J Androl 2006 Jul; 8: 393–403)*

Keywords: allograft; germ cells; spermatogenesis; testis; xenograft

1 Introduction

Substantial efforts have been made to establish optimal *in vitro* models simulating spermatogenesis with hopes of male germ cells completing meiosis and spermatid elongation in experimental conditions. In particular, because of the increasing popularization of the intracytoplasmic sperm injection technique, researches on *in vitro* maturation of germ cells have been increased [1–6].

Using immunodeficient animals as an incubator for ectopic grafting of immature testes or testicular tissues

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provides a promising new approach not only for providing a new *in vitro* model essential for future research on testicular function and germ cell development in human and large animals, but also for the preservation of testicular function and maintenance of endangered species [7, 8]. In comparison with other *in vitro* systems, this technique makes it easier to simulate the microenvironment and to standardize the experimental condition, and it brings better reproducibility. Since the foremost work was reported in 2002 [7], this technique has been used in various species to differentiate immature germ cells into functional sperm [9–16].

In the present study, we performed a large scale allografting experiment using immunodeficient mice as recipients and neonatal mouse testes as donor tissues to provide systematical information on graft survival, differentiation and stage-specific gene expression of germ cells in different developmental stages. Based on the allografting experiment, we then performed xenografting with testes from human fetuses as donor tissues to test the practicability of this animal model in human studies.

2 Materials and methods

2.1 Animals and donor tissues

Animals were purchased from the Experimental Animal Center of the Southern Medical University in Guangzhou, China and maintained in a controlled environment with 12 h : 12 h light : dark cycles from 06:00 to 18:00. The Scientific Committee of the Peking University Shenzhen Hospital approved the use of animals for experimental purposes. The Ethics Committee of Peking University Shenzhen Hospital approved all animal procedures and the use of human tissues for research purposes. All correlative operations, including the acquisition of donor testicular tissue, the recipient castration and the grafting, were cautiously performed inside a laminar-flow sterile hood.

Male immunodeficient BALB/c-nu/nu mice (7–12 weeks old and weighing between 22 and 28 g) were used as recipients, and bred in individually ventilated cage systems (Shanghai Tianhuan Science Develop CO. Ltd., Shanghai, China) with ^{60}Co-sterilized fodder and autoclaved tap water available ad libitum and handled in accordance with standard operating procedures for specific pathogen-free animals. Eighty nude mice were used for both allografting and xenografting experiments.

Kunming mice used as donor animals for the allografting study were conventionally bred. Donor testes were isolated from neonates (between postnatal days 1 and 2, killed by decapitation) and collected in sterile saline containing penicillin (1 000 U/mL) and streptomycin (1 mg/mL) immediately before grafting. Before grafting, testes were prepared by making an incision in testicular capsule to expose the seminiferous cords. A total of 450 testes (average wet weight 1.61 ± 0.64 mg) from 225 male mice were used for the allografting experiment.

As donor tissues for the xenografting study, testes from two spontaneously aborted human fetuses stillborn at 20 and 26 weeks gestational age were collected and kept in an ice-cold sterile D-MEM (Promega, Madison, WI, USA) medium containing penicillin (1 000 U/mL) and streptomycin (1 mg/mL) and immediately transported to the laboratory. Testicular tissues for grafting were prepared as previously described [11].

2.2 Allografting study

Seventy-five recipient mice were divided into 16 groups (namely, group I for 3 days, group II–XII for 1–11 weeks and group XIII–XVI for 3–6 months of grafting time) with six recipients in group I–IX and three in group X–XVI. Castration and grafting procedure was performed as previously described with each castrated nude mouse receiving six neonatal mouse testes [9].

Groups of recipient mice carrying neonatal mouse testes were killed by cervical dislocation at different time intervals and grafts were removed together with the conjoint skin and directly observed under microscope on the structure inside grafts through the capsule. Grafts were then collected and the status of graft survival, development and gene expression was assessed.

2.2.1 Morphological and histological observations

A small fragment of the collected graft tissue was taken for an immediate morphological observation of germ cells in fresh tissues. Briefly, a small piece of graft was picked with a sterile needle and disaggregated in a phosphate-buffered saline solution containing 1 mg/mL collagenase IV for 5–10 min and observed under a phase-contrast microscope.

For histological analysis, graft tissues were generally processed and stained with haematoxylin and eosin (HE) and periodic acid-Schiff (PAS). For comparison, reciprocal samples of testicular tissues from normal donor mice were also parallel analyzed.

2.2.2 Determination of gene expression

A fraction of graft tissue was immediately stored at –80ºC for molecular biological determination. Stage-specific expression of deleted in azoospermia-like (*DAZL*), testis-specific protein 57 (*Tsp57*), phosphoglycerate kinase 2 (*Pgk2*), testis-specific protein kinase 1 (*TESK1*) and protamine-2 (*Prm2*) mRNA in grafts were determined using reverse transcriptase-polymerase chain reaction (RT-PCR). For comparison, the expression of these genes in normal donor animals of relevant ages was parallel determined. Primer sets (listed in Table 1) were designed using the Primer 3.0 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) program according to the mRNA sequences, with the specificity determination by alignment analysis using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) on the Internet and synthesized by Shengong Biotech Company (Shanghai, China).

Grafts and normal testicular tissues were homogenized in TRIZOL reagent (Mrcgene, Cincinnati, OH, USA) and total RNA were prepared according to the manufacturer's instructions. After determination of RNA concentration by spectrophotometric method, RT-PCR was performed using the one-step RT-PCR kit (Finn-zymes, Espoo, Finland) in a 50 μL total reaction volume with 1 μg RNA, 5 IU avian myelobastosis virus (AMV) reverse transcriptase, 2 IU Taq DNase, 5 μL of 10 × polymerase chain reaction (PCR) buffer, 1 μL of 10 mmol/L dNTPs Mix, 1.5 μl of 50 mmol/L $MgCl_2$ and 2 μL of 5 pmol of each primer. RT reaction was run at 48ºC for 45 min and PCR was initiated with a predenaturation step at 94ºC for 2 min followed by 40 cycles of 30 s at 94ºC, 30 s at the annealing temperature (59ºC for *DAZL*, 54ºC for *Tsp57*, 58ºC for *Pgk2*, 60ºC for *TESK1* and *Prm2*) and 30 s at 72ºC. A final extension for 7 min at 72ºC completed the PCR. The absence of contaminants was routinely checked using negative controls in which RNA samples were replaced with sterile water. Glyseraldehyde-3-phosphate dehydrogenase (GAPDH) was also analyzed to confirm equal quantities of RNA. PCR products were electrophoretically resolved in parallel with size marker on 1.2% agarose gel. The sequence confirmation of *DAZL*, *Tsp57*, *Pgk2*, *TESK1* and *Prm2* PCR product was performed with sequencing of target fragments by INVITROGEN (Guangzhou, China).

2.3 Xenografting study

For the xenografting experiment, five castrated nude mice were used, with two receiving testicular tissues from the 20-week fetus and three receiving testicular tissues from the 26-week fetus. Immediately after castration, two skin incisions of 4–5 mm were made on either side of the dorsal midline and four testicular tissue fragments per recipient were subcutaneously placed into the back skin of recipient mice.

Host animals receiving human testicular tissues were killed by cervical dislocation on day 116 and 135 after the grafting procedure. After documenting the volume and weight, grafts were processed for histological analysis. Testicular tissue fragments from both fetal testes before grafting were also parallel analyzed.

3 Results

3.1 Allografting study

3.1.1 Evaluation of graft growth

All 75 recipient mice receiving neonatal mouse testes survived until the scheming time and were in good condition at the time of graft collection. Gradual progresses of grafts in all experimental groups were macroscopically observed and survival of grafted testes was confirmed by measuring the weight of grafts (Table 2).

At the time of graft collection, back skin of each recipient was stretched and photographed to document the survival and growth of grafts (Figure 1A). A clear

Table 1. Primer information.

Gene	GenBank Access No.	Primer		Amplicon (bp)
		Sense (5'-3')	Antisense (5'-3')	
DAZL	NM010021	ggtgtgtcgaagggctatg	gcggtggcatctggtagt	352
Tsp57	AY251192	cacagtaaagccctgtgcaa	atgaatttgggcaaatgagc	171
Pgk2	BC061054	gtgtgggccctgaagtagag	tttggctccaccaaggatag	353
TESK1	NM011571	gggatggagatggagtgaga	acaggacgacttgagggttg	251
Prm2	BC049612	agcccagagcgcgtagag	ggcctggggaggcttagt	241
GAPDH	BC083149	acccagaagactgtggatgg	ccaccctgttgctgtagcc	424

Table 2. Recovery of grafting procedure and graft weight in comparison with testis weight in normal donors. [a]Time after the grafting procedure for grafts and age for normal donor. [b]Total numbers of grafted testes were 36 for groups I–IX and 18 for groups X–XVI. —, Not measured.

Group	I	II	III	IV	V	VI
Time[a]	3 days	1 week	2 weeks	3 weeks	4 weeks	5 weeks
Number of collected rafts	25	26	35	36	34	33
Recovery (%)[b]	69.44	72.22	97.22	100	94.44	91.67
Graft weight (mg, mean ± SD)	2.64 ± 1.33	7.12 ± 4.87	9.41 ± 3.98	12.32 ± 8.49	21.56 ± 8.58	27.15 ± 10.00
Testis weight (mg) of normal donor (n = 6, mean ± SD)	2.27 ± 0.64	5.67 ± 0.67	9.13 ± 0.75	14.90 ± 3.08	20.87 ± 2.35	76.55 ± 4.70
Group	VII	VIII	IX	X	XI	XII
Time	6 weeks	7 weeks	8 weeks	9 weeks	10 weeks	11 weeks
Number of collected rafts	35	33	35	16	18	11
Recovery (%)	97.22	91.67	97.22	88.89	100	61.11
Graft weight (mg, mean ± SD)	25.82 ± 9.79	24.17 ± 11.35	27.88 ± 14.16	26.35 ± 11.08	27.98 ± 14.22	26.63 ± 18.91
Testis weight (mg) of normal donor (n = 6, mean ± SD)	101.81 ± 6.35	97.10 ± 14.27	106.11 ± 17.87	—	—	121.35±3.78
Group	XIII	XIV	XV	XVI		
Time	3 months	4 months	5 months	6 months		
Number of collected rafts	18	16	18	16		
Recovery (%)	100	88.89	100	88.89		
Graft weight (mg, mean ± SD)	27.86 ± 8.29	23.38 ± 9.68	29.83 ± 17.06	13.84 ± 9.52		
Testis weight (mg) of normal donor (n = 6, mean ± SD)	166.35 ± 0.45	—	—	—		

layer of capsule covered the graft tissue and vascular tissues of each recipient extensively extended into the graft. Graft masses significantly increased with advancing time, 1.61 ± 0.64 mg at the time of grafting up to 27.15 ± 10.00 mg after 5 weeks of the grafting procedure. Seminiferous tubule-like structures inside grafts were clearly seen through the capsule after putting a whole graft under light microscope (Figure 1B).

3.1.2 Observation of germ cells in fresh graft tissues

Observations on enzymatically disaggregated graft tissues from the 7-week group revealed a presence of all stages of germ cells, including round spermatids with acrosome and typical mouse sperm with falciform heads (Figure 1C, D).

3.1.3 Histological observations

At the grafting time, seminiferous cords of testis by day 1 or 2 post-partum contained only two types of cells: gonocytes as the only germ cell type and somatic cells,

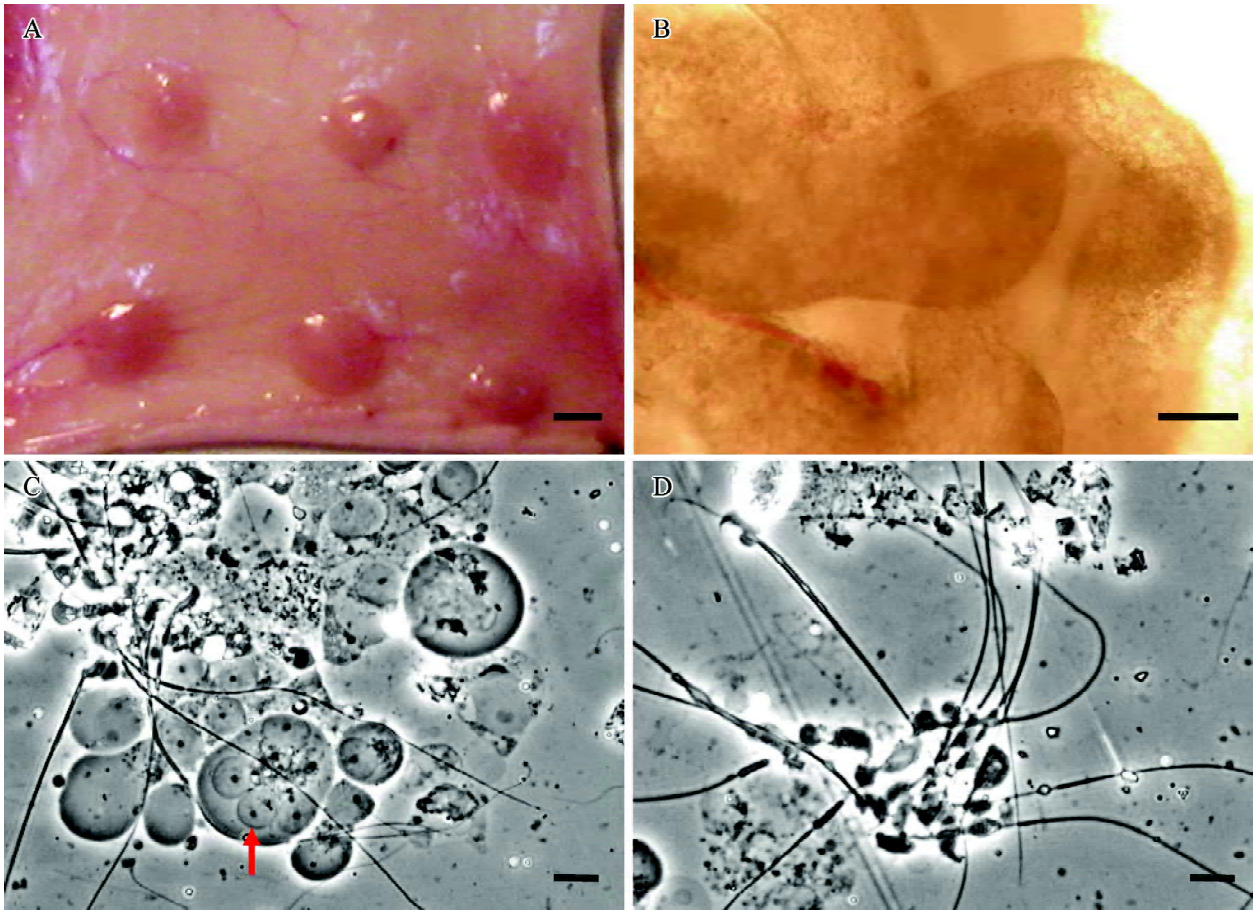

Figure 1. Observation by graft collection. (A): Graft growth in a 7-week group host mouse (bar: 5 mm). (B): Vascular tissue and seminiferous tubule-like structure inside graft observed by putting a whole graft under light microscope (bar: 50 μm); × 400. (C): Observation of germ cells including round spermatids (red arrow) in fresh graft (bar: 10 μm); × 400. (D): Typical mouse sperm with falciform head in fresh graft (bar: 10 μm): × 400.

Sertoli cells (Figure 2A). *In vivo*, meiotic germ cells first appeared in a developing mouse testis at the day 9 post-partum and their numbers increased with time; around day 15, nearly 100% of seminiferous cords developed lumen and a few round spermatids were found; by day 17, a visible number of round spermatids were observed and they significantly increased by day 23; elongate spermatids were first found in seminiferous tubules by day 27; 5 weeks after birth, the appearance of seminiferous epithelium looked fairly close to the adult stage with the germ cells arranging in an ordered sequence from the basement membrane to the lumen: spermatogonia lying directly on the basement membrane, following by spermatocytes, spermatids and sperm as one progressing toward the lumen. These observations were in accordance with the previous histological and ultrastructural analyses [17]. With grafts, pachytene spermatocytes were first found in the 1 week group (but actually at day 9 of their development because testes from 2-day neonatal mice were used at the grafting time, Figure 2B); a large number of spermatocytes could be seen in the 2-week group and tubule lumen were was developed as well (Figure 2C); in the 3-week group round spermatids were found in many tubules (Figure 2C); in the 4-week group only a few elongate spermatids could be seen and the number was significantly increased in the 5-week group (Figure 2D); in the 7-week group the seminiferous epithelium looked like in an intact adult (Figure 2E), in which cell combinations of all XII stages were observed by PAS stained samples [18]. From 8 weeks af-

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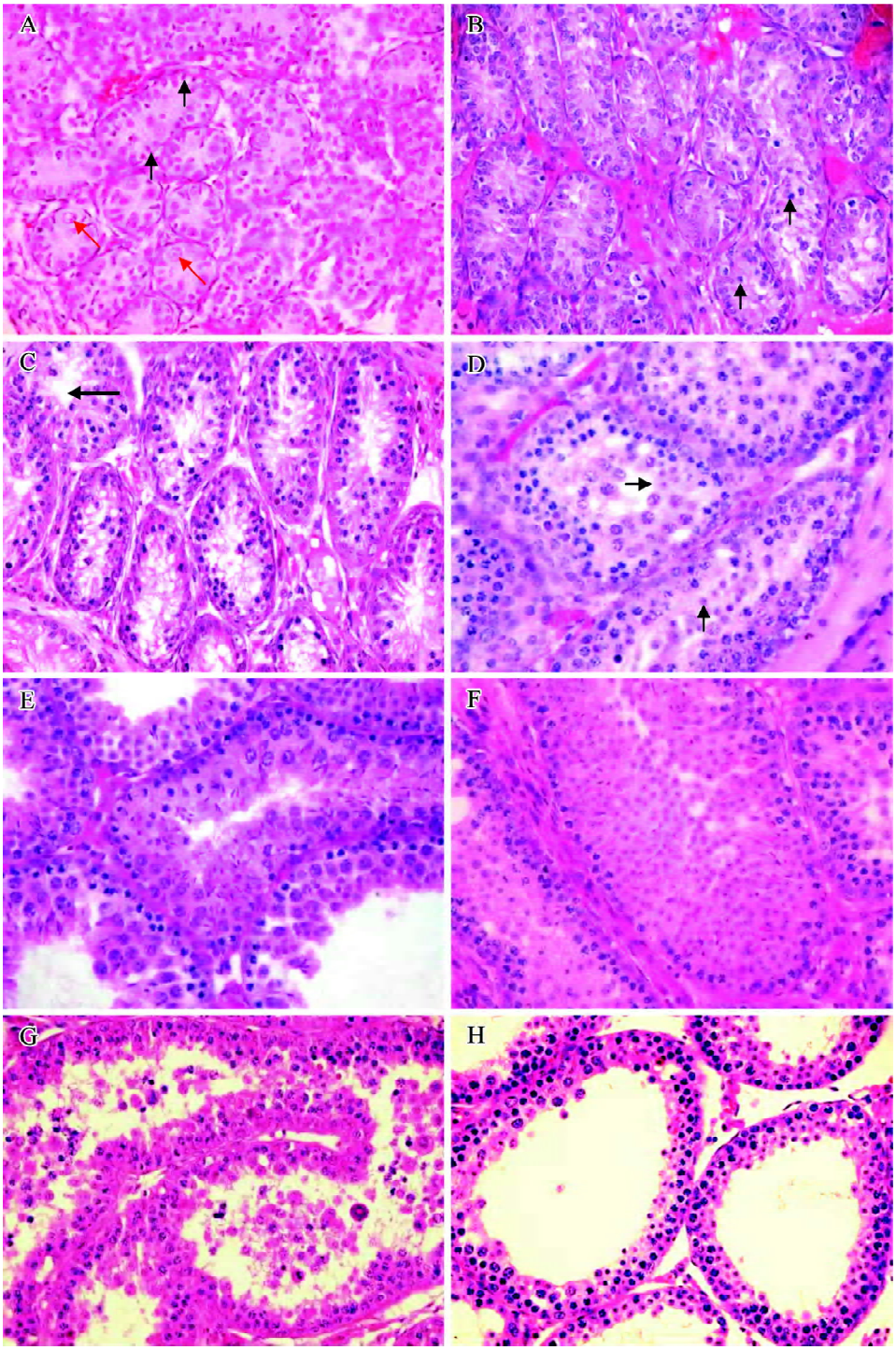

Figure 2. Histological observations on allografts (haematoxylin and eosin stained). (A): Testis from neonatal mouse of 1 day post-partum containing two types of cells in seminiferous cords (approximately 50 μm in diameter), gonocytes (red arrow) and Sertoli cells (black arrow); × 400. (B): Graft of 1-week group showing spermatogonia and a few spermatocytes (black arrow); × 400. (C): Graft of 2-week group displaying luminal development (black arrow) and increasing number of spermatocytes; × 400. (D): Graft of 3-week group showing round spermatids (black arrow); × 400. (E): Graft of 5-week group displaying complete spermatogenesis; × 400. (F): Graft of 7-week group exhibiting an adult-like structure of seminiferous tubule with diameter of approximately 150 μm; × 400. (G): A large number of germ cells casting off in graft of 3-month group; × 400. (H): Graft of 4-month group displaying degradation of seminiferous epithelium; × 400.

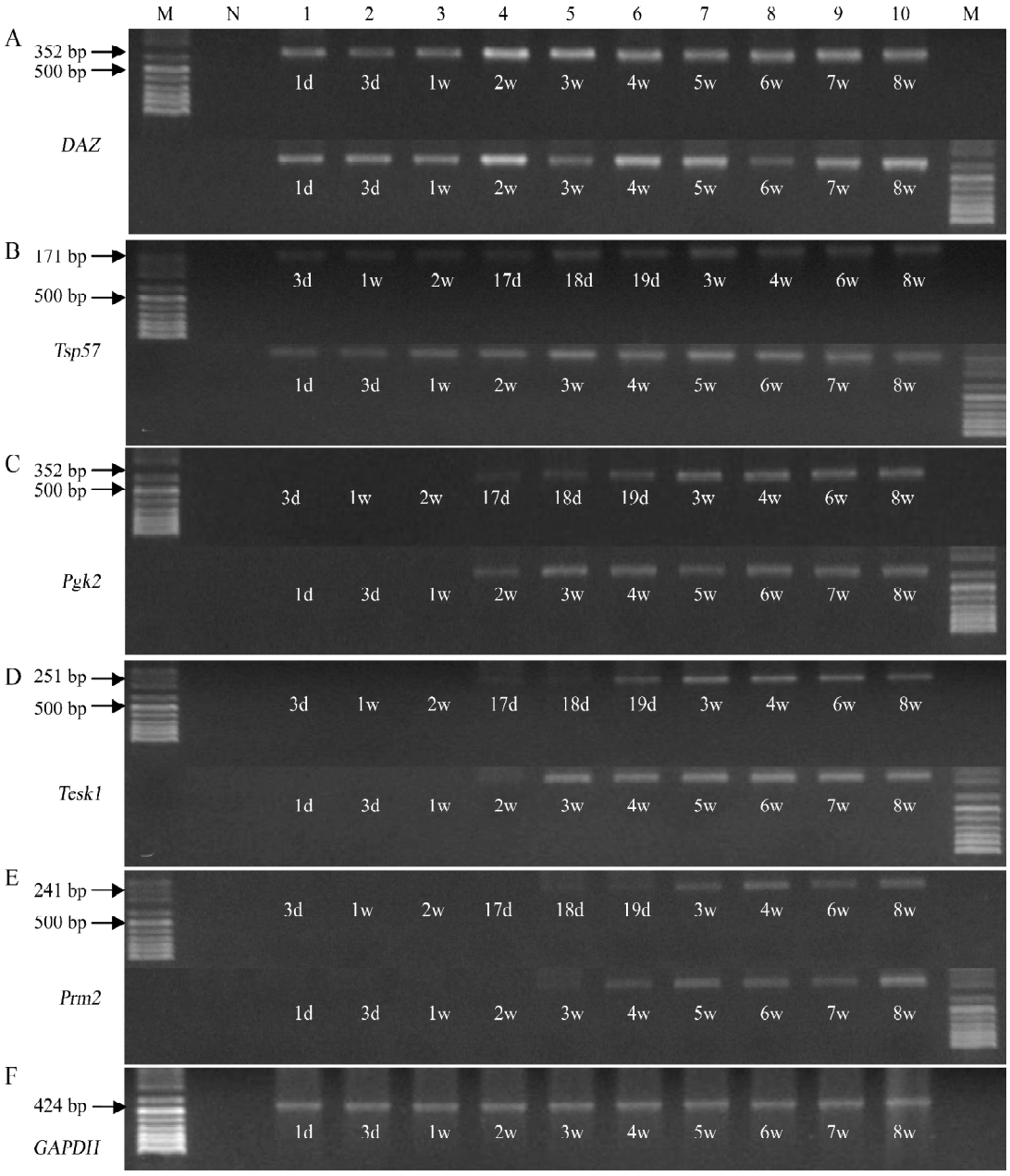

Figure 3. Expression of *DAZL*, *Tsp57*, *Pgk2*, *TESK1* and *Prm2* mRNA in grafts and normal donor testes. (A)–(E): Presenting *DAZL*, *Tsp57*, *Pgk2*, *TESK1* and *Prm2* reverse transcriptase-polymerase chain reaction (RT-PCR) products, respectively. (F): *GAPDH* RT-PCR products of grafts as RNA loading control. Lanes 1–10 (top): Testicular tissues from normal donor mice. Lanes 1–10 (bottom): Grafts obtained at different time intervals. Lanes M: DNA ladder (bands from bottom to top: 1 000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100 and 50 bp). Lane N: negative control.

ter grafting, a significant degradation of seminiferous epithelium was found (Figure 2G-H), which was in accordance with the previous study [10].

3.1.4 Determination of gene expression

The electrophoretogram in Figure 3 summarizes the RT-PCR results showing the expression of *DAZL*, *Tsp57*,

Pgk2, *TESK1* and *Prm2* mRNA in grafts obtained from group I to group IX (namely, 3 days to 8 weeks after grafting) and reciprocal testicular tissues of normal donor mice. In normal donors, *DAZL* mRNA was detected in testes from day 1 post-partum till 8 weeks; *Tsp57* mRNA was of small amount in 1 day–2 week mouse testes, increased from 17 days and reached a high level from 3 to 8 weeks; *Pgk2* mRNA was in trace amounts at 9 days and significantly increased from 3 weeks; a slight expression of *TESK1* mRNA was observed at 14 days and increased from 18 days; *Prm2* mRNA appeared in mouse testes from the day 21 after birth and increased with time. The expression of the five genes in grafts showed a similar trend as seen in normal donors, which exhibited good correlation with histological observations. Sequences of *DAZL, Tsp57*, *Pgk2, TESK1* and *Prm2* PCR products both from normal donors and grafts showed consistency with target regions.

3.2 Xenografting study

3.2.1 Assessment of graft survival

Of the five nude mice that received xenografts, three died possibly as a result of infection that occurred during housing between day 4 and 8 after grafting. Two mice, one with grafts from the 20-week fetus and another with grafts from the 26-week fetus, survived until they were killed at day 116 and 135, respectively. They were in good condition at the end of the experiment except for a mild skin ulcer at the surface of the grafting sites. Two grafts were obtained from each of the two hosts; namely, two with approximately 2 mm in diameter and 10 mg and 12 mg in wet weight and another two with approximately 2.5 mm and 3.5 mm in diameter and 20 mg and 25 mg in wet weight, respectively.

3.2.2 Histological evaluation

By microscopic observation, HE stained testicular tissue samples from 20- and 26-week fetuses at the time of grafting showed almost the same structure and cell composition. Testicular cords with approximately 60 ± 15 μm in diameter were dispersedly embedded in surrounding interstitial tissues of the testis. Inside cords, germ cells, gonocytes and somatic cells, immature Sertoli cells distributed in a random arrangement. Bigger and round germ cells displayed loose and light karyoplasms with one or two nucleoli, whereas smaller sized immature Sertoli cells displayed more compact nuclei and gathered together in a group surrounding the germ cells. The arrangement of long axes of oval nuclei of Sertoli cells was of a random manner as the testicular cord had not yet developed lumen. There were voluminous interstitial tissues, in which fibroblasts and relatively bigger Leydig cells with plenty acidophilic cytoplasm were observed (Figure 4A, B, E).

Both grafts from day 116 and 135 of grafting time showed similar histological appearance. Some of the quondam testicular cords in the 135-day group, approximately 10%, had developed lumina becoming seminiferous tubules with significantly increased diameters up to 80 ± 25 μm. Seminiferous tubules were lined by the seminiferous epithelium consisting of two types of cells: Sertoli cells and germ cells. Sertoli cells with abundant curtain-like cytoplasm were distributed around the circumference of seminiferous tubules and their oval nuclei arranged themselves in a relative order with their long axes upright to the base. Most of germ cells had migrated and located between the Sertoli cells and the basal lamina. In contrast to Sertoli cells, long axes of germ cell nuclei were parallel to the base. And some of them had changed their shapes from round to elliptic and karyoplasms had become compact with shrunken or disappeared nucleoli. A few germ cells had the appearance of spermatogonia, which were all surrounded by the curtain-like cytoplasm of Sertoli cells forming a clear niche structure (Figure 4C, D, F).

4 Discussion

In the allografting study, a significant increase in tissue volume and weight as well as in tubule diameter occurred 4 weeks after the grafting procedure, indicating germ cell development and Sertoli cell proliferation, confirmed by histological observation [12]. Graft weight continued to increase throughout the 5-week period reaching a plateau at 5 weeks.

The time of appearance of all kinds of germ cells in grafts, including spermatogonia, spermatocytes, round and elongate spermatids and sperm, was comparable with that in intact donors.

Because a normal course of spermatogenesis required a highly concerted genic net involving a series of specific genes regulating and controlling the process in a cooperative manner, five genes in different developmental stages were chosen for the study. *DAZL* gene transcribed predominantly in testes and was detectable 1 day after birth [19]. Targeted disruption of *DAZL* in mouse

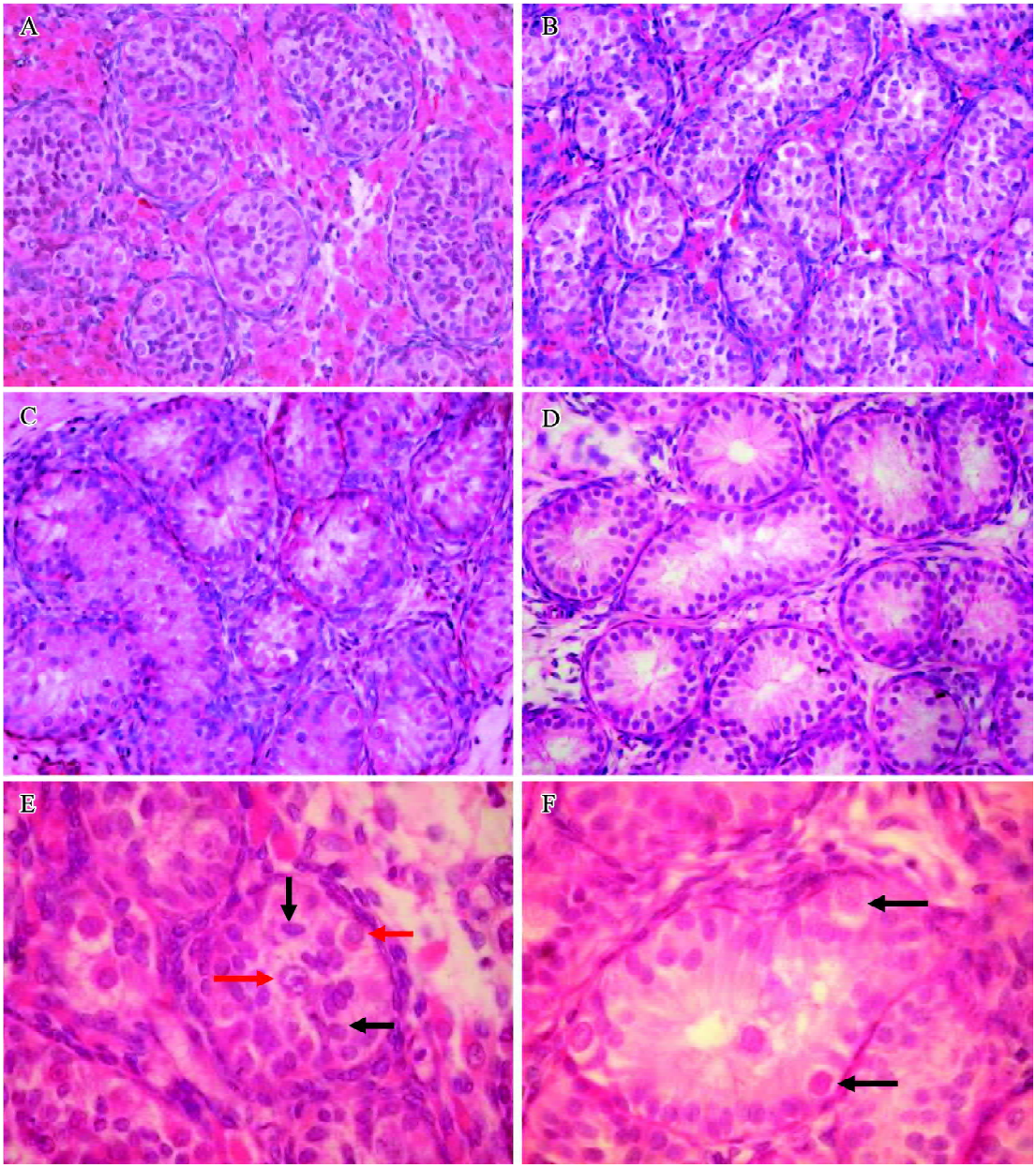

Figure 4. Histological observations on xenografts (haematoxylin and eosin stained). (A): Testicular tissue from the 20-week fetus; × 400. (B): Testicular tissue from the 26-week fetus; × 400. (C): At 116 days after the grafting procedure, cells in seminiferous cord showed in (A) migrating towards the basement membrane; × 400. (D): At 135 days after the grafting procedure, testicular tissue showed in (B) having luminal development; × 400. (E): High power objective microphotograph of (B) clearly showing dispersed distribution of cells in seminiferous cords, gonocytes with bigger and round nuclei (red arrow) and immature Sertoli cells with smaller and oval nuclei (black arrow); × 630. (F): High power objective microphotograph of (D) clearly showing an ordered arrangement of cells in seminiferous tubules, germ cells locating at the basement membrane and Sertoli cells with more mature state surrounding the germ cells (black arrow) forming a niche structure; × 630.

resulted in infertility in both male and female mice [20]. The expression of *Tsp57* mRNA was highly restricted to the testis and it possibly played an important role in the postmeiotic phase of germ cell differentiation [21]. *Pgk2* gene coincidently initiated with the onset of meiosis in male germ cells and specifically expressed in meiotic and postmeiotic germ cells [22, 23]. *TESK1* mRNA, a stage-specific marker for the meiosis of germ cells specifically

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expressed in rat and mouse testicular germ cells, especially in round spermatids [24, 25]. *Prm2* specifically expressed in the haploid stage of differentiating male germ cells and played an essential role in the process of chromatin condensation during spermiogenesis [26, 27]. These genes in grafts of different developmental stages showed a consistent expression pattern with those in intact donors, which had also a good coincidence with previous studies [19–27] and with the mouse cyclogeny reported by Bellve *et al.* [17].

Although the germ cells in allografts behaved similarly in development and gene expression as in intact donors, hypogenesis of seminiferous tubules was found by all developmental stages of grafts, unlike the *in vivo* situation as seen in control animals. A notable degradation of grafts was observed after 5 weeks and the damage to the seminiferous epithelium increased with grafting time confirmed by graft weight and histological observations. Increasing numbers of degraded seminiferous tubules were observed with grafting time. These results were in accordance with the previous study, in which follicle stimulating hormone (FSH) level in recipient sera was systematically measured and the increasing damage of seminiferous epithelium with grafting time was interrelated with the increase in FSH [10].

In the xenografting study using immature human testes as donor tissues, we showed that testicular tissues from human fetuses survived and further developed in castrated nude mice confirmed by four- to five-fold increases in graft weight; seminiferous cords developed into seminiferous tubules with increased diameter and lumina development; Sertoli cell differentiation was accelerated, as was previously suggested that the FSH concentration in recipient mice initiated functionality of these cells earlier than would be normally seen in physiological condition [7]; randomly distributed Sertoli cells and germ cells had migrated to the basement membrane, which is suggested to have occurred at puberty *in vivo* [28]; germ cells at the basal lamina were surrounded by Sertoli cells with their curtain-like cytoplasm forming a niche-like structure, which was supposed as the fundamental factor of spermatogenesis [29, 30]. In addition, several Leydig cells with plumpish acidophilic cytoplasm presented in interstitial tissue of the fetal testis before grafting and they were found to be atrophic after grafting. This seemed to be a coincident, or could be explained with the evolution of man [28]. According to the above observations, the development stage of grafts seems to be as in a testis of a prepuberal level, indicating a shortening of the time required for human testicular maturation as previously observed on other mammals [7, 11]. In two recent studies using human testicular tissue from infertile, transsexual and tumor patients [31, 32], xenografts showed limited survival, which was in agreement with previous study using mature testes [9]. The different fate between mature and immature testicular tissues in xenografting might be because (i) steroidogenesis in grafts from mature tissues was very low in comparison with prepubertal donor tissues that could supply normal to elevated levels of androgens to the castrated donors [5, 9, 11, 31], and (ii) immature tissues might have a better ability to survive periods of ischaemia or might be more effective for angiogenesis in the host than the adult tissue [32].

However, these data from the present experiment provided only limited information due to the restriction in obtaining donor tissues from human. Although the animal model has been successfully used in a slow maturing primate, rhesus monkey [11], it remains still elusive as to whether it can be used for human investigation because sexual maturity lasting for 12–14 years in human is much longer than in rhesus monkey (3–4 years). Further investigations on xenografting with immature human testicular tissues for a longer period of grafting time are needed.

Findings of present studies provide additional information for the practicability of the animal model. Ectopic allografting has lead to a stepwise development of immature germ cells with the initiation of gene expression comparable to that *in vivo*. The best time for retrieval of mouse sperm from grafts is 5–7 weeks after grafting before a significant damage to seminiferous epithelium occurrs. An accelerated development of immature human testicular cells might be achieved by ectopic xenografting of human testes, which could provide a novel strategy for germline preservation and for omitting malignant relapse and be valuable for studying the early stages of human spermatogenesis [31, 32].

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