Grafting Period and Donor Age Affect the Potential for Spermatogenesis in Bovine Ectopic Testis Xenografts

Jonathan A. Schmidt, Jeanene M. de Avila, and Derek J. McLean

Department of Animal Sciences and Center for Reproductive Biology, Washington State University, Pullman, Washington 99164

ABSTRACT

Bovine testis tissue xenografts contain elongating spermatids 6 mo after grafting. The percentage of seminiferous tubule cross sections with elongating spermatids at the time of graft removal varies depending on donor age and rarely exceeds 10%. These data indicate significant changes are occurring to bovine testicular cells during the first weeks of life. The objective of this research was to xenograft testis tissue from multiple ages of bull calves for 24 or 36 wk in order to gain a better understanding of early bovine testis development. Testis tissue from 1-, 2-, 4-, and 8-wk-old calves was grafted onto the backs of castrated immunodeficient mice. Testis tissue from all donor ages grew, differentiated, and produced testosterone and elongating spermatids. Testis tissue grafts from 1- and 8-wk-old calves had elongating spermatids in greater than 5.5% of seminiferous tubule cross sections at the time of graft removal regardless of grafting period. Four-week-old donor tissue never had more than 5.2% of seminiferous tubule cross sections with elongating spermatids. Extending the grafting period from 24 to 36 wk resulted in an increase in the percentage of seminiferous tubule cross sections with elongating spermatids from 2% to 10% in 2-wk donor tissue. These data demonstrate that both donor age and grafting period may be important factors regulating the maturation of bovine testis xenografts, indicating that intrinsic differences exist within testis tissue at these donor ages. These data provide the framework for further study of bovine spermatogenesis using ectopic testis xenografting.

INTRODUCTION

The development of the testis is initiated in the embryo and continues after birth until puberty is achieved and sperm capable of fertilizing an egg are present in an ejaculate. This complex process requires precise and coordinated differentiation of somatic cells and germ cells that undergo meiosis and differentiate into sperm. Testis development, puberty, and continual production of sperm in adults are under the control of various cells and organs, including the hypothalamus, the pituitary gland, and the somatic cells of the testis. The period of time it takes for testis development and puberty varies between species, requiring only weeks in rodents, months in most livestock, and years in primates [1]. Investigation of cellular species, requiring only weeks in rodents, months in most pituitary gland, and the somatic cells of the testis. The period of continual production of sperm in adults are under the control of different differentiation into sperm. Testis development, puberty, and differentiation of somatic cells and germ cells that undergo meiosis and complete spermatogenesis are present in an ejaculate. This framework to understand the complex processes resulting in sperm production.

Multiple factors critical for testis development and sperm production have been identified in rodents with the use of targeted gene deletions and genomic approaches [2–4]. Similarly, spermatogonial stem cell transplantation has been used to identify factors regulating spermatogonial stem cell biological activity in multiple rodent species [5]. In contrast, few models or techniques exist to investigate testis development and spermatogenesis in large mammalian livestock species. Research in large animals using spermatogonial stem cell transplantation has focused on technique development and subsequent fertility [6, 7]. Transplantation of nonrodent germ cells into the testes of immunodeficient mice results in spermatogonial stem cell colonization; however, germ cells do not differentiate, and complete donor-derived germ cell differentiation has not been observed [8]. Another technique, ectopic testis tissue grafting, is an approach to produce sperm in testis tissue after removal from the donor animal [9]. In this technique, small pieces of prepubertal donor testis tissue are grafted subcutaneously on the backs of immunodeficient mice. The grafted testis tissue undergoes growth and cellular differentiation, resulting in complete germ cell differentiation. Ectopic xenografts using donor testis tissue from a variety of donor species, including mice, pigs, goats, bulls, monkeys, hamsters, and cats, undergo complete germ cell differentiation [9–14]. Haploid germ cells isolated from mouse testis homografts have been used with intracytoplasmic sperm injection to generate offspring [15]. Testis tissue xenografting can be used as a novel tool to investigate basic mechanisms of sperm production in large animals. Additional applications include the generation of genetically modified sperm [10], germ line preservation (in livestock, endangered species, and humans), a model to identify causes of infertility, cryopreservation of gametes [9, 13], and use as a model for the development of novel sterilization techniques.

The period of pubertal development in bulls lasts from birth to approximately 36 wk of age, at which time sperm can be found in the ejaculate. In the bull calf testis, spermatogonia are first observed at 16 wk of age. Meiotic cells are present at 20 wk of age, and elongating spermatids are present at 28 wk of age. By 32 wk of age spermatozoa are present in the seminiferous tubules [16]. The mechanisms regulating somatic and germ cell differentiation leading to complete spermatogenesis in the bull are relatively uncharacterized. The development of alternative techniques to study bovine spermatogenesis would accelerate and improve the understanding of this complex process.

Prepubertal bull testis tissue will grow, differentiate and undergo complete germ cell differentiation when grafted onto castrated immunodeficient mice [10, 11, 17]. Bull testis tissue from 2-, 4-, 8-, 12-, and 16-wk-old animals was grafted onto mice for 24 wk and elongating spermatids were present in...
all donor age grafts except for the 2-wk donor [11]. In contrast to other species, acceleration of germ cell differentiation was not observed in bovine testis tissue xenografts [9–11]. Two-week donor tissue had the highest potential for growth, whereas 8-wk donor tissue had the greatest potential to produce elongating spermatids [11]. Although informative, these experiments did not allow testis grafts to remain on the mice at endpoint times past 24 wk. This resulted in analysis of testis grafts from 2-wk donors that were the equivalent of 26 wk old at removal, which is earlier than when elongating spermatids are present in the testes of intact bulls.

Spermatogenesis in bovine testis xenografts is inefficient in that the maximum percentage of seminiferous tubule cross sections in the grafted tissue that produce elongating spermatids is 11% [10, 11]. This value was from 8-wk donor testis tissue that was grafted for 24 wk. Thus, it is possible that younger donor testis tissue could produce more elongating spermatids if it remained on the mouse for a longer time. Rathi and colleagues [17] xenografted 2-wk donor tissue and concluded that the low efficiency of spermatogenesis in bovine testis grafts is due to an initial loss of germ cells and an impairment of germ cell differentiation. However, these researchers only evaluated testis tissue from 2-wk donors. It is possible that differences in testis tissue of donors of different ages could result in differences in graft development and ultimately the efficiency of germ cell differentiation.

From these data, we developed the hypothesis that spermatogenesis in grafted bovine testis tissue requires the tissue to remain on the mouse until the tissue reaches the age equivalent to that at which elongating spermatids appear in bull testes in vivo. Furthermore, we believe that this technique could provide a novel method to evaluate differences in the early (prior to 8 wk of age) establishment of spermatogenesis in the bovine testis by evaluating the development of testis grafts from donors within this developmental period.

The objective of the following research was to investigate the donor age and grafting period effects on bovine ectopic testis tissue xenografting, in order to gain a better understanding of early bovine testis development. Additionally, this research could further identify the most optimal donor age and grafting period for compete germ cell differentiation in bovine testis grafts. To do this, testis tissue from several donor ages was grafted onto mice and ultimately removed at either 24 or 36 wk postgrafting. This experimental design would provide conditions in which the somatic and germ cells in bovine testis tissue could differentiate for a period of time sufficient to ensure complete germ cell differentiation in the grafted tissue. We hypothesized that testis tissue that was grafted and remained on mice to a tissue age that was equivalent to the donor age when elongating spermatids are present in the testis would contain a higher percentage of seminiferous tubules with elongating spermatids than tissue that was removed at an earlier time point. Furthermore, we hypothesized that any differences observed in germ cell differentiation over time between donor ages could be due to innate differences in developing testes.

**MATERIALS AND METHODS**

**Materials**

All reagents, unless otherwise stated, were purchased from Sigma (www.sigmaaldrich.com). Immunodeficient NCr nude mice (Taconic, www.taconic.com; CrlTac:NCr-Fox(Fnu1nu)) were raised under normal conditions and were fed a standard rodent chow ad libitum. Immunodeficiency in this mouse strain stems from an abnormal thymus due to homozygosity of the autosomal recessive nude gene (nmu/nmu). Angus cross bull calves were castrated at 1, 2, 4, and 8 wk of age. The Washington State University Animal Care and Use Committee approved all animal procedures.

**Tissue Collection.** Testis tissue samples were obtained from at least 4 bull calves per donor age. Bull calves were castrated using standard techniques, and testes were immediately placed in Hanks Balanced Salt Solution (HBSS) on ice or dissected and placed in Bouin fixative for 4 h at 4°C followed by dehydration and storage in 70% ethanol. Testis tissue for grafting was maintained in HBSS. Testis tissue was detunicated, and parenchyma tissue was dissected away and cut into 3–5 mm (2–3 mm) pieces and returned to ice until the time of grafting.

**Ectopic Testis Tissue Xenografting.** Immediately after donor testis dissection, four pieces of testis tissue were ectopically grafted onto 2- to 4-mo-old adult castrated immunodeficient mice. Briefly, mice were anesthetized with ketamine (0.1 mg/kg body weight [BW]) and xylazine (0.5 mg/kg BW) in sterile physiological saline. A ventral median incision was made in the skin and the testes were removed and the peritoneum and skin were sutured closed using absorbable suture (Ethicon, www.novartis.com). After castration, mice were placed in ventral recumbence, four incisions were made in the skin on the back of the mice, donor testis tissue was inserted at each site, and the incisions were sutured closed. The mice were allowed to recover and were returned to their cages.

**Histological Analysis of Donor Grafts.** Grafts were removed at 24 and 36 wk after grafting. Mice were killed by CO2, inhalation and cervical dislocation. Grafts were removed, weighed, and fixed in Bouin fixative at 4°C for 4 h followed by dehydration in 70% ethanol. Grafts were washed in xylenes, blocked in paraffin, and sectioned at 8 μm. After sectioning, slides were deparaffinized, rehydrated, and stained with hematoxylin and eosin. The grafts were evaluated using light microscopy, and digital images were captured with a Leica DFC 280 digital camera and a Leica DME compound microscope (Leica Microsystems Imaging Solutions Ltd., www.leica-microsystems.com) at 400× magnification.

Graft cross sections were evaluated to determine the percentage of seminiferous tubule cross sections with spermatagonia, meiotic germ cells, elongating spermatids, or no germ cells. Spermatagonia were identified by the presence of highly condensed elongating nuclei and cellular basal location within the seminiferous tubule. Meiotic germ cells were identified by the presence of diffuse nuclear staining. Elongating spermatids were identified by the presence of highly condensed elongating nuclei. The percentage was determined by dividing the total number of tubule cross sections within a graft cross section containing a germ cell type in all grafts on a given mouse by the total number of tubule cross sections in the same graft cross sections from that mouse. This calculation prevented unreasonable bias between grafts that had different numbers of seminiferous tubule cross sections, which might arise if graft percentages were simply averaged. Seminiferous tubule diameter was determined for all round seminiferous tubule cross sections within a graft cross section and averaged per mouse. The largest center cross section from recovered grafts was evaluated for germ cell differentiation and seminiferous tubule diameter.

**Recipient Mouse Analysis.** At the time of killing, blood was collected from the recipient mice by cardiac puncture. Serum was isolated from the blood and assayed for testosterone as an indicator of testis tissue viability. Testosterone concentrations were determined by RIA (DSL-400; Diagnostic Systems Laboratory Inc., www.dslabs.com). Vesicular glands were also removed and weighed to determine the bioactivity of the testosterone produced by the donor graft.

**Statistical Analysis.** Testis tissue grafts and serum from mice with functional grafts were analyzed. Grafts were considered functional if they contained active germ cell differentiation and were found on recipient mice with at least 0.5 ng/ml of serum testosterone and vesicular glands that weighed at least 250 mg. Due to the variability of the number of seminiferous tubule cross sections per graft, the percentages of seminiferous tubule cross sections with each type of differentiating germ cell and seminiferous tubule diameters were determined per mouse. Serum testosterone concentration, mouse vesicular gland weight, and mouse serum testosterone concentration were determined using the Duncan test for significance.
Differences between treatments were considered significant at $P \leq 0.05$. Data are presented as the mean ± SEM.

### RESULTS

**Testis Tissue Graft Weight, Seminiferous Tubule Diameter, Serum Testosterone Concentration, and Vesicular Gland Weight**

Testis tissue from 1-, 2-, 4-, and 8-wk-old bull calves was grafted onto castrated immunodeficient mice and removed 24 or 36 wk later. Recipient mice received testis tissue at four sites; however, grafted tissue was not always recovered from every site. As shown in Table 1, graft recovery ranged between 64% and 100%, with the lowest recovery of testis tissue being the 4-wk donor tissue grafted for 24 wk. Recovered testis tissue grafts were categorized as functional based on active germ cell differentiation in seminiferous tubules and testosterone production resulting in mouse blood serum testosterone concentration of 0.5 ng/ml or higher and vesicular gland weight of 250 mg or higher. There was no difference in the percent of grafts recovered or functional status between the donor ages or graft periods ($P > 0.05$; Table 1). Functional and nonfunctional testis tissue grafts were often present on the same recipient mice. All analyses reported for the percentage of seminiferous tubule cross sections containing differentiating germ cells are from functional testis tissue grafts only.

Testis tissue graft weight was measured at the time of removal to determine if donor age and/or graft period had an effect on testis tissue growth. Overall, 1-wk donor testis tissue had the greatest growth rate of all the donor ages (Fig. 1A). Regardless of graft period, 1-wk donor grafts were significantly ($P < 0.05$) larger than other donor ages. No difference in testis graft weight was observed between 24 and 36-wk graft periods when all graft weights were pooled. However, 2-wk donor testis that was on the mice for 36 wk was more than twofold larger ($P < 0.05$) than grafts recovered 24 wk after grafting. These data indicate that testis tissue from 1-wk donors have more growth potential than testis tissue from the other donor ages.

Seminiferous tubule diameter was measured to determine the developmental potential of testis tissue from different aged donors that were grafted for different grafting periods. There was no significant difference ($P \geq 0.05$) in seminiferous tubule cross section diameter between donor ages in the 36-wk grafting period (Fig. 1B). Two-week donor grafts grafted for 24

### Table 1. Experimental graft data.

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<th>Donor Age (wk)</th>
<th>Graft period (wk)</th>
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<th>No. of grafts recovered (%)</th>
<th>No. of mice analyzed</th>
<th>No. of functional grafts (%)</th>
<th>No. of complete spermatogenesis grafts (%)</th>
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* Total grafts removed divided by the total number of grafts grafted.

b Total functional grafts divided by total recovered grafts.

c Percentage of functional (as a percentage of recovered) grafts with elongating spermatids.

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FIG. 1. Growth analysis of 1-, 2-, 4-, and 8-wk functional donor testis tissue grafts removed at 24 or 36 wk after grafting. **A** Average weight of testis tissue grafts. **B** Average seminiferous tubule diameter in testis tissue grafts. Asterisks (*) indicate a significant difference ($P < 0.05$) between the same donor age at different grafting periods. Different letters above bars within grafting periods indicate significant differences between donor ages within the grafting period. Data are presented as the mean, with error bars indicating ± SEM.
wk had significantly smaller seminiferous tubule cross section diameters than other donor ages grafted for 24 wk or 2-wk donor grafts grafted for 36 wk. In contrast, testis tissue from 4-wk-old donors grafted for 24 wk had seminiferous tubules in which the diameter was larger ($P < 0.05$) than the testis tissue from the same donors grafted for 36 wk (Fig. 1B).

To determine if graft Leydig cells were functional, testosterone concentration and bioactivity in the serum of mouse recipients were measured. Serum testosterone concentrations in mice grafted with 2-wk donor testis tissue were significantly higher ($P < 0.05$) than mice grafted with 4- and 8-wk donor testis tissue at the 36-wk grafting period (Fig. 2A). Recipient mouse serum testosterone was not different in recipient mice grafted with testis tissue from any other group or combination (Fig. 2A). The vesicular glands from recipient mice were weighed to assess the bioactivity of the testosterone produced by the grafts. No significant difference ($P \geq 0.05$) was observed in vesicular gland weights in mice that were grafted with different aged donor testis tissue during the specific grafting periods (Fig. 2B). However, mice with 2- and 8-wk-old donor testis that remained on the mice for 36 wk had larger ($P < 0.05$) vesicular glands than mice grafted with donor testis tissue from the same donor age for only 24 wk. Additionally, when pooled, mice with grafts that were removed after 36 wk had larger ($P < 0.05$) vesicular glands than mice with grafts that were removed after 24 wk (Fig. 2B).

**Histological Analysis of Testis Tissue Grafts**

Histological evaluation of donor tissue and grafted tissue was conducted to determine if donor age or grafting period had any effect on the ability of germ cells in the grafted tissue to differentiate and undergo spermatogenesis. Seminiferous tubule cross sections of each graft were evaluated for the presence of spermatogonia, meiotic germ cells, or spermatids. All seminiferous tubule cross sections from the largest graft cross section were analyzed.

The seminiferous tubules of testis tissue from donor bull calves at each age contained gonocytes, Sertoli cells, and interstitial cells (Fig. 3). Recovered bovine testis tissue grafts characterized as functional from all donor ages had complete spermatogenesis as evidenced by the presence of elongating spermatids (Table 1). In contrast to previous reports, 2-wk donor tissue had complete germ cell differentiation after 24 wk of grafting (Fig. 4, C and D). Similar results were observed for 1-wk donor tissue (Fig. 4, A and B).

The extent of germ cell differentiation in testis grafts was evaluated to determine if testis tissue from different aged donors grafted for different periods had different proportions of seminiferous tubule cross sections undergoing complete germ cell differentiation. Overall, 2-wk donor tissue grafted for 24 wk had the poorest germ cell differentiation (Fig. 5). These grafts had lower percentages of seminiferous tubule cross sections containing spermatogonia and spermatocytes and a higher percentage that were Sertoli cell only compared to 1- and 8-wk donor tissue grafted for 24 wk. Within the 36-wk grafting period, it appeared that 4-wk donor tissue had the poorest germ cell differentiation (Fig. 5). These grafts had a lower percentage of seminiferous tubule cross sections containing spermatocytes and elongating spermatids compared to other donor tissue in the 36-wk grafting period. Comparison of donor ages across different grafting periods indicated the only difference between periods was for the 2-wk donor tissue (Fig. 5). Two-week tissue grafted for 36 wk had a higher percentage of seminiferous tubule cross sections with spermatogonia, spermatocytes, and elongating spermatids and a lower percentage of seminiferous tubule cross sections with Sertoli cells only (Fig. 5). Overall, it appeared that elongating spermatid production in 8-wk-old donor testis grafts were the most consistent across time. Furthermore, a higher percentage of recovered grafts from 8-wk donor testis contained complete germ cell differentiation (Table 1).

In contrast to previous studies [10, 11], donor testis tissue from 1- and 2-wk-old bull calves grafted for 24 wk was able to undergo complete germ cell differentiation (Table 1 and Fig. 4). Analysis of testis tissue grafts from these donors in this study indicated that germ cell differentiation was accelerated in tissue from donors at these ages because elongating spermatids were present in testis grafts after a 24-wk grafting period. This correlates to a 2- and 3-wk acceleration in the first round of spermatogenesis normally observed in bull testis at 28 wk of age.

**DISCUSSION**

Testis differentiation requires formation of seminiferous tubules in conjunction with somatic cell maturation and germ
cell differentiation. Spermatogonia are first present in bull testes around 16 wk of age, the same age at which Sertoli cell proliferation slows and these cells begin to differentiate [16]. Prior to this maturation, changes in the germ cell population are subtle but likely critical for the establishment of the testicular environment that will lead to efficient spermatogenesis. Ectopic testis tissue xenografting provides a model to investigate differences in immature testis tissue from bull calves of different ages. The donor ages and grafting periods used in previous work with bovine testis xenografting do not cover the ranges of possible donor ages or the potential time required for grafts to fully mature. The objective of the current study was to investigate the donor age and grafting period effects on bovine ectopic testis tissue xenografting. We hypothesized that testis tissue that was grafted and remained on mice to a tissue age that was equivalent to the donor age when elongating spermatids are present in the testis would contain a higher percentage of seminiferous tubules with elongating spermatids than tissue that was removed at an earlier time point. Furthermore, we hypothesized that any differences observed...
in germ cell differentiation over time between donor ages could be due to innate differences in developing testes.

In the present research, testis tissue from 1-wk-old donors had the greatest ability for growth as indicated by graft size, regardless of grafting period (24 or 36 wk). The ability of testis tissue from the other donor ages (2, 4, 8 wk) to grow was not significantly different. However, growth of 2-wk donor tissue continued during the longer grafting period.

All grafted testis tissue had the ability to produce testosterone. Vesicular glands were larger at 36 wk, most likely due to a longer period for testosterone to stimulate gland growth. Serum testosterone levels were highest in mice with 2-wk donor testis tissue grafts at 36 wk postgrafting. This could be due to the increased graft size and, potentially, Leydig cell number of these grafts. Mice with 8-wk donor tissue 36 wk after grafting had the lowest serum testosterone levels; however, intratesticular testosterone levels were adequate to maintain spermatogenesis because of the presence of complete germ cell differentiation in the grafts. Overall, androgen production is more consistent than graft growth across donor ages and graft periods. This is most likely due to innate characteristics of Sertoli, Leydig, and germ cells. Leydig cells appear to be more flexible as to what donor age is most appropriate for the endocrine environment of a castrated mouse. Sertoli cells and subsequently germ cells appear to be more sensitive to donor age and the mouse endocrine environment.

Testis tissue from all donor ages was able to undergo complete germ cell differentiation during both grafting periods. Few differences were observed in the percentage of seminiferous tubule cross sections with spermatogonia, Sertoli cells only, or meiotic germ cells. The combinations of donor age and grafting period that resulted in the poorest production of differentiating germ cells were 2-wk donors grafted for 24 wk and 4-wk donors grafted for 36 wk. These combinations had smaller percentages of seminiferous tubule cross sections with differentiating germ cells and a higher percentage of seminiferous tubule cross sections with Sertoli cells only. The best combination of donor age and graft period was 2-wk donors grafted for 36 wk. The percentage of seminiferous tubule cross sections with elongating spermatids in these grafts exceeded 10%; however, neither this combination nor any of the others exceeded previously published percentages [10, 11].

Germ cell differentiation was more consistent in 8-wk donor grafts regardless of grafting period compared to other donor ages. Several possible reasons for this exist. First, 8-wk donor tissue could be at a stage requiring maximal gonadotropin support. Grafting onto castrated recipients would provide a graft with maximum FSH concentrations. Additionally, once germ cell differentiation has become asynchronous, seminiferous tubules with complete spermatogenesis are always present in a testis. This may account for why a 36-wk grafting period results in comparable spermatogenesis to a 24-wk period. Analysis of donors of other ages at a single time point may increase the possibility that the germ cells have not differentiated to elongating spermatids during the first round of spermatogenesis. Furthermore, observation of tissue in the second round of spermatogenesis prior to asynchronous germ cell differentiation could result in underestimations of elongating spermatid production.

Four-week donor testis underwent complete germ cell differentiation regardless of grafting period. However, extended grafting times appeared to significantly reduce the growth potential of grafts as well as their ability to maintain germ cell differentiation.

Thirty-six-week testis grafts from 2-wk donors had a higher percentage of seminiferous tubule cross sections with germ cells compared to the 24-wk grafts, which had a higher percentage of seminiferous tubule cross sections with Sertoli cells only. The differences between 2-wk donor testis tissue grafted for 24 wk or 36 wk are substantial. It has been reported that the low efficiency of sperm production in grafted bovine testis tissue is due to an initial deficit in germ cells and impaired germ cell differentiation [17]. These researchers concluded that 2-wk donor tissue grafted for 24 wk had a considerably smaller percentage of seminiferous tubules with differentiating germ cells than the same tissue grafted for 36 wk. However, the effect of grafting period for donor ages other than 2 wk was not reported. In the present research, individual donor testis tissue was maintained for both 24 and 36 wk, and no obvious trends regarding survival of tissue
from specific donors is evident. Additionally, all recipient mice were the same age. Therefore, it is unlikely that these differences are due to the donor testis tissue or the recipients. Indeed, no difference in germ cell differentiation after 24 and 36 wk of grafting for 1- and 8-wk-old donor testis tissue (in contrast to the results for 2- and 4-wk donor tissue) supports the conclusion that developmental differences in testis tissue from 1 to 8 wk of age can have dramatic effect on germ cell differentiation and sperm production.

One-week donor bull testis tissue had not been previously used for ectopic testis tissue grafting. Our results indicate that 1-wk donor tissue undergoes complete germ cell differentiation by 24 wk of grafting. Interestingly, production of elongating spermatids in the 1-wk grafts was similar to 8-wk donors at both 24 and 36 wk.

The research presented here is important for several reasons. First, this data supports previous research indicating that donor age may play a significant role in the efficiency of spermatogenesis in bovine testis xenografts. Furthermore, this research also suggests that the period of time in which testis tissue remains on the mouse is critical for completion of germ cell differentiation and is most likely dependent on donor age. A second important finding of this research is that donor tissue from 2- and 4-wk-old bull calves has inconsistent development across grafting periods. This indicates that the developmental period between 1 and 8 wk of age may be important for the initial development of the testis. Slight differences in endocrine environments could have a more dramatic effect on testis tissue of these ages. Somatic and germ cells in testes from 1- and 8-wk-old bull calves could be less sensitive to the endocrine environment of recipient mice than testis tissue from 2- and 4-wk-old donors. It is possible that cells in testis tissue from 1-wk-old bull calves have not adequately differentiated and 8-wk tissue is too differentiated to be negatively affected by the change in environment. Nevertheless, these findings indicate that intrinsic differences between testis tissue at 1, 2, 4, and 8 wk of age exist, and these differences can dramatically influence the efficiency of bovine ectopic testis tissue grafting. Finally, the presence of elongating spermatids in 1- and 2-wk-old donors at 24 wk postgrafting indicates that the first round of spermatogenesis is accelerated in bovine ectopic testis tissue grafts. This is contrary to previous reports analyzing the pattern of meiotic germ cell populations in 4-wk donors over time [10], yet in agreement with results from pig and monkey xenografts [9, 12]. The exact mechanism initiating accelerated spermatogenesis is unknown. It is possible that spermatogenesis is initiated earlier in bovine xenografts; however, it is also possible that the mouse endocrine environment stimulates a faster rate of germ cell differentiation. Regardless of the mechanism, grafting of 1- and 2-wk-old bull testis tissue onto mice is an uncoupling event resulting in accelerated spermatogenesis.

Ectopic bovine testis xenografting is a promising tool for the study of bovine spermatogenesis. In order for the success of this technique to be maximized, the combination of donor age and grafting period that maximizes the percentage of seminiferous tubules with elongating spermatids must be identified. The present research indicates that 2-wk donors grafted for 36 wk is the best combination examined to date; however, 1- and 8-wk-old donors grafted for either 24 or 36 wk can also be used successfully. Intrinsic differences in testis tissue at the different donor ages may be responsible for the variation observed in graft survival and development. Elucidation of factors responsible for these differences could provide means to develop new contraceptive techniques or to increase the fertility of livestock animals. Finally, these data provide a framework for further research using ectopic bovine testis tissue grafting as a model to study bovine neonatal testis development and adult spermatogenesis.

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