RESEARCH ARTICLE

Human oocyte-like germ cells derived in vivo from testicular tissue of a Klinefelters' patient: A case report

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Abstract

The combination of testicular biopsy and intracytoplasmic sperm injection procedures has assisted potentially azoospermic men with Klinefelters' syndrome to have children. Approximately two-thirds of the time, a testicular sperm extraction procedure performed on a nonmosaic Klinefelter 47,XXY patient fails to isolate sperm. In the case report described herein, both wet preparation and histological examinations confirmed typical cellular morphologies associated with Klinefelter testicular tissue, including no spermatozoa. It did however, observe the unusual presence of oocyte-like cells representing a unique, theoretical in vivo transformation of pluripotent stem cells, probably spermatogonial, into primordial germ cells and gametes of the opposite sex, as previously documented only in the experimental mouse model.

Keywords: testicular tissue, Klinefelter syndrome, oocytes, stem cells

Introduction

Klinefelters' syndrome is genotypically characterized by a 47,XXY karyotype in men. The extra X chromosome of Klinefelter males is believed to cause azoospermia via germ cell degeneration,[1] although the condition may be incomplete as residual spermatogenesis can be present in some seminiferous tubules.[2] The testicular histology of adult XXY mice exhibits small seminiferous tubules with varying degrees of intraepithelial vacuolization and the absence of germ cells.[3] Furthermore, hypertrophy and hyperplasia of Leydig cells are observed in the interstitium.[3] The hyperactivity of Leydig cells seen in XXY mice suggests that the changes in the endocrine milieu observed in Klinefelter syndrome is not due to impaired Leydig cell function.[4] Although sperm is rarely seen in ejaculates, 27-42% of nonmosaic Klinefelter men possessing a 47,XXY karyotype may yield spermatozoa upon testicular biopsy.[5-8] These sperm are capable of fertilizing

mature oocytes by intracytoplasmic sperm injection and creating genetically healthy babies.[5,9-12]

The epiblast of developing embryos creates primordial germ cells which are the precursors to spermatogonia and oogonia. In culture, primordial germ cells can be induced to differentiate into pluripotent embryonic germ cells in the presence of various growth factors. Investigators have previously shown that human testicular tissue derived pluripotent stem cells are capable of inducing ecto-, meso- and endodermal tissue formation.[13] Despite genotypic differences of germ cells of males and females, both types of germ cells share the same progenitors, namely, primordial germ cells. The ultimate fate of XY male germ cells is dependent on environmental signaling in the gonad.[14] Several studies have demonstrated that the progeny of primordial germ cells/gonocytes, derived from human and murine spermatogonial stem cells, can be reprogrammed into embryonic stem-like cells *in vitro* without transgene manipulation,[15-19] revealing the remarkable plasticity of spermatogonial stem cells. The existence of spermatogonial stem cells in the human testis has been characterized in vitro.[20] The purpose of this case report is to provide observational documentation that testicular stem cells can be reprogrammed to form primordial germ cells and oocytes in a Klinefelter male.

Methods

A 29-year old healthy, married Caucasian male was determined to not have sperm in his ejaculate upon semen analysis (i.e., azoospermia), consistent with the infertile status of the couple. His endocrine profile revealed normal serum testosterone and prolactin levels (290.7 ng/dL and 7.9 ng/mL, respectively), while his FSH and LH levels were abnormally elevated (23.2 mIU/mL and 32.0 mIU/mL, respectively). Upon further testing, he was determined to have a 47,XXY karyotype indicative of a clinical diagnosis of nonmosaic Klinefelter syndrome. The patient presented with a tall, thin athletic build (6'2", 190 lbs), normal intelligence and motor skills, and no other apparent genetic deficits.

Desiring a fertile outcome, the patient pursued Urological treatment. A micro-surgical testicular sperm extraction procedure was performed on both testicles in August 2008. The testicular tissue was evaluated directly in the operating room by an experienced Embryologist (MCS), and a piece of testis biopsy from each side was fixed in Bouin's solution and sent out for histological evaluation. The seminiferous tubules were selectively dissected in HEPES-buffered human tubal fluid medium (Irvine Sci., Santa Ana, CA) supplemented with 5% human serum albumin (Irvine Sci.), and a brief preliminary assessment was made for the presence of sperm before the next specimen was procured. Further detailed analysis of the shredded cellular debris was performed in an IVF laboratory using an inverted microscope under 400X magnification, as previously described.[21]

As this case report did not involve a clinical study, investigational review board (IRB) approval or exemption was not sought. However, a standard informed consent was signed by the patient, confirming his approval that testicular biopsy outcome data may be published with standard anonymity as to patient identification, consistent with ethical research expectations.

Results

Some variation in seminiferous tubular morphology was noted, with many tubules appearing vacuolated and most testis tissue having shortened, less convoluted sections. Furthermore, in most cases the tubules were difficult to shred using fine 27ga needles, having a somewhat sclerotic tendency upon stereomicroscopic dissection. Cellular dispersion of the dissected testis biopsy tissues was not robust, and few, if any, spermatogenic cells were detected. Additionally, sertoli cells and some other small cells (approximately10 μm round) were identified. The pathology report described germ cell aplasia with extensive fibrosis of the tubules with Leydig cell hypertrophy (Fig. A), which is consistent with Klinefelter's syndrome. Yet, other in vivo observations were not so typical. The initial search of the cellular milieu/debris failed to report the presence of any sperm, as did further detailed assessments. However, early in the evaluation process it was semi-jokingly said that "there is no sperm, but appears to be egg-like cellular masses". As it turned out, that is all that was found of significance. The first of which was the 130μm egg surrounded by a distinct zona pellucida layer, shown below (Fig. B1). Upon detailed analysis in the IVF laboratory, hundreds of zona pellucida-free oocyte-like primordial germ cells (ranging in size from 20-70μm) were observed, from a particular biopsy region.

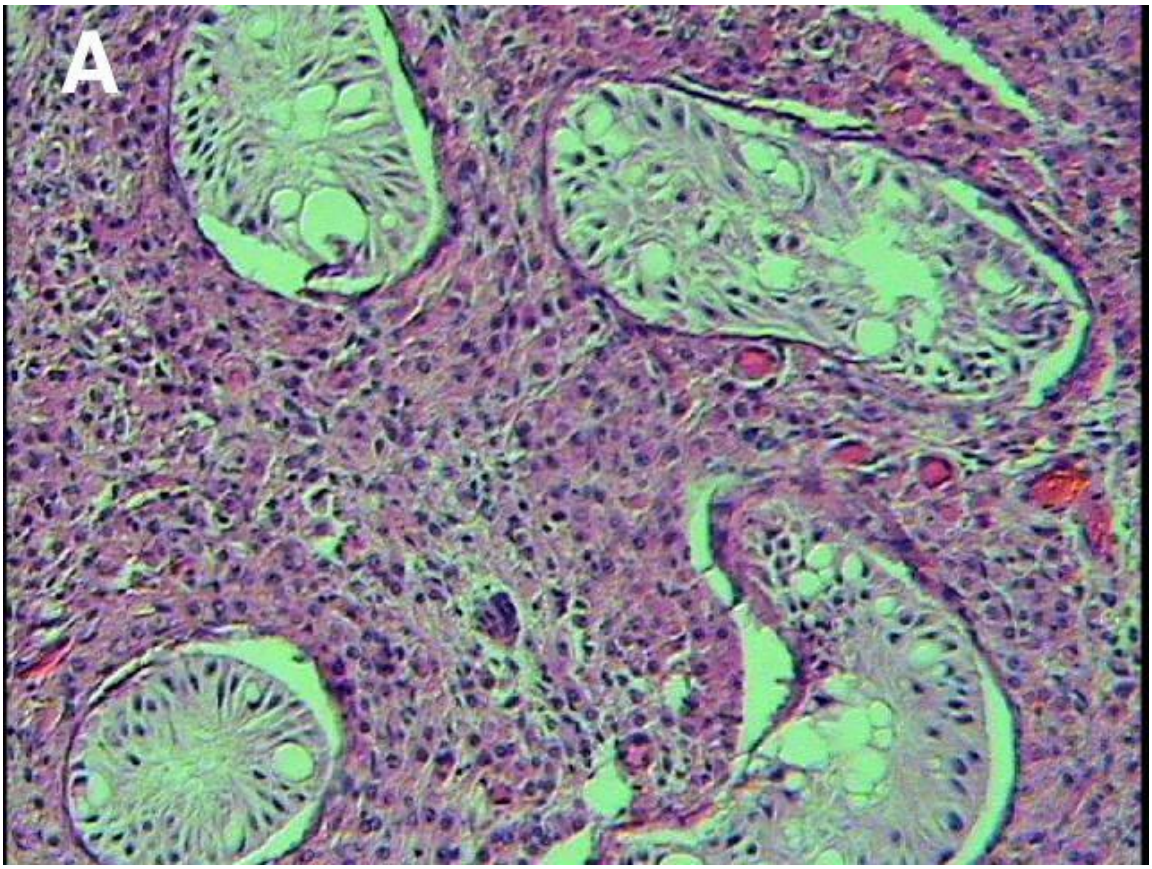

Figure A. Histological examination of this Klinefelter patient exhibits cellular hypertrophy and hyperplasia of leydig cells in the interstium between seminferious tubules displaying germ cell aplasia.

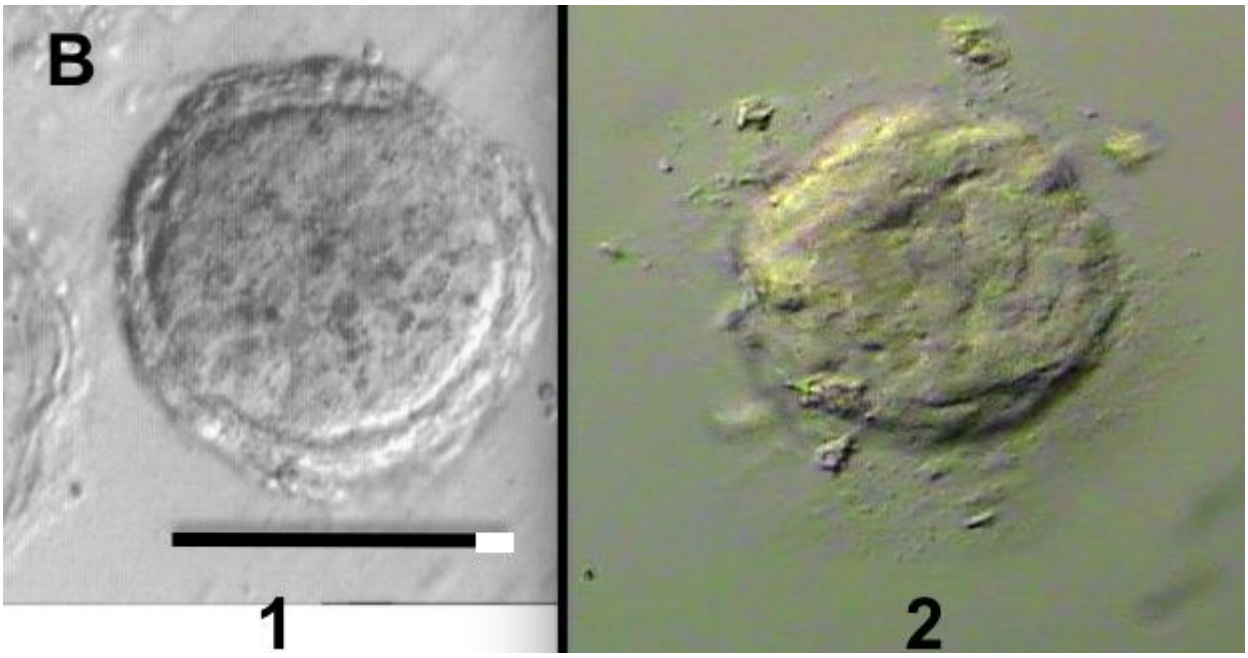

Figure B. The fully developed oocyte-like cell found in the dissected testis celluar milleu of the Klinefelter, 47 XXY male is the size of a mature egg (B1; scale: equals 100µm) and is surrounded by an apparent zona pellucida. The textured appearance of the cytoplasm seemed usual, until we contrasted it to the documented texture of ooplasm in an oocyte experiencing osmotic shrinkage during vitrification solution exposure (B2). In comparison, the testicular-derived oocyte looks remarkably similar to a mature human oocyte undergoing vitrification solution exposure.

Discussion

Eppig and O'Brien (1996) first demonstrated that mouse spermatogonial stem cells possess the potential to be reprogrammed into oocyte-like cells and high quality oocytes when culture conditions were optimized using a 3D culture system with a supportive cell feeder layer. [22] Subsequently, XY embryonic stem cells were proven capable of differentiating into oocytes in culture.[23,24] Several mouse studies have now demonstrated that spermatogonial stem cells can also revert back to pluripotency as embryonic stem-like cells under certain culture conditions.[25] In fact, male embryonic stem cells and induced pluripotent stem cells have been shown to differentiate into primordial germ cell–like cells in vitro.[26] Upon transplantation into mouse testes, murine primordial germ cell–like cell lines have proven capable of forming fully functional sperm.[27] Hayashi and coworkers (2012) also found that female mouse stem cells can be induced to differentiate into primordial germ cell–like cells, which when aggregated in reconstituted ovaries, do exhibit epigenetic reprogramming and meiotic potential in vitro.[28]

By addressing questions about the role of specific genes in early germ cell development and the interaction between germ cells and supporting somatic cells, science has begun to unravel the mysteries of cellular differentiation.[29] Observational and biochemical tests are able to assess

some cellular properties, as shown with human spermatogonial stem cells[20] and testicular induced pluripotent stem cells.[13] However, these assays are inadequate to judge whether the cells would support the normal development of functional gametes. Murine pluripotent stem cells were first induced into primordial germ cell-like cells when aggregated with somatic cells of female embryonic gonads, the precursors for adult ovaries. When transplanted under the ovarian bursa of mice, these cellular aggregates formed germinal vesicle stage oocytes within a month. Hayashi's group (2013) has determined that these stem cell-derived GV oocytes can be matured into viable eggs capable of fertilization with spermatozoa to obtain healthy and fertile offspring.[28] Wang and coworkers (2012) have also shown that mouse spermatogonial stem cells can be converted into oocyte-like cells in culture which are similar in size to normal mature murine oocytes.[25] They have further exhibited that oocyte-specific markers are expressed and that the oocytes can produce embryos through parthenogenesis. Interestingly, both the Y- and X-linked testis-specific genes in mouse spermatogonial stem cell derived-oocytes are significantly down-regulated or turned off, while oocyte-specific X-linked genes are activated.[25] Although human stem cell investigations have yet to attain the success of the murine model forming functional gametes, it is feasible that a Klinefelter male with an extra X chromosome could experience a genomic upregulation resulting in oocyte-like germ cells being produced in an isolated region containing viable stem cells.

It is widely accepted that isolated pockets of spermatogenesis can occur in seemingly azoospermic Klinefelter men,[2] therefore it is possible that spermatogonial stem cells were present to transform into oocyte-like cells in vivo. Since there were no hermaphroditic indicators in this patient and histological analysis failed to reveal ovarian parenchyma, a rare ovotestis condition was ruled out. In turn, the likely explanation is that testicular spermatogonial, or perhaps fibroblasts, in this patient underwent a pluripotent stem cell transformation into primordial germ cell-like cells and the formation of oocyte-like cells. Because of the unexpected, surprising and unbelievable nature of this finding, photo documentation was the only verification conducted of this unique event, with no attempt being made to cryopreserve the tissue for subsequent testing. Unfortunately, prior to 2009 our laboratory was not applying vitrification technology or oocyte freezing which could have safely preserved the cellular integrity of those cells for additional genetic and biochemical examination. In turn, we acknowledge our failure to take advantage of this rare scientific discovery. Therefore, we were unable to assess the potential viability of these oocyte-like germ cells, nor do we have any biochemical evidence to confirm whether and how this unique phenomenon occurred in a Klinefelter male. However, other scientific investigators have previously shown that human testis tissue can produce induced pluripotent stem cells in vitro.[13,15,16] This case study does provide in vivo evidence that human pluripotent stem cells can potentially be induced and programmed to create gametes of either sex in vitro and in vivo.[30,31]

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