Published in final edited form as:
J Androl. 2010 ; 31(1): 86–94. doi:10.2164/jandrol.109.008367.

LDHC THE ULTIMATE TESTIS SPECIFIC GENE

Erwin Goldberg[1,3], **Edward M. Eddy**[2], **Chongwen Duan**[1], and **Fanny Odet**[2]

[1]Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208

[2]Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709

Abstract

Lactate dehydrogenase C (LDHC) was, to the best of our knowledge, the first testis specific isozyme discovered in male germ cells. In fact, this was accomplished shortly before “Isozymes or isoenzymes” became a field of study. LDHC was detected initially in human spermatozoa and spermatogenic cells of the testes by gel electrophoresis. Immunohistochemistry was used to localize LDHC first in early pachytene primary spermatocytes with an apparent increase in quantity following meiosis to its final localization in and on the principle piece of the sperm tail. After several decades of biological, biochemical and genetic investigations we now know that the lactate dehydrogenase isozymes are ubiquitous in vertebrates, developmentally regulated, tissue and cell specific and multi-functional. Here we will review the history of LDHC and the work that demonstrates clearly that it is required for sperm to accomplish their ultimate goal, fertilization.

Keywords

Lactate dehydrogenase C; glycolysis; male fertility; testis; sperm

DISCOVERY OF LDHC: THE BEGINNING OF A JOURNEY

The isozyme concept owes its development to Clement Markert (Markert and Appella, 1961) who was one of the first to recognize the significance of multiple molecular forms of enzymes, and since this time, isozymes have been extensively studied or used as markers for normal and abnormal cell function. One of the earliest studied and best examples is the lactate dehydrogenase (LDH) enzyme family which catalyzes the interconversion of pyruvate to lactate with the concomitant oxidation/reduction of NADH to NAD^+ (Everse and Kaplan, 1973). The first evidence of the presence of multiple forms of LDH was found using the technique of protein electrophoresis (at this time in a starch matrix) elaborated by Smithies (1959) coupled with cytochemical visualization of LDH activity (Allen, 1961; Dewey and Conklin, 1960). We learned that LDH consists of A and B subunits that assemble into homo- or heterotetramers that are distributed in the body in combinations reflecting the metabolic requirements of different tissues and consistent with the catalytic properties of the isozymes. For example, LDHA is most abundant in skeletal muscle where oxygen deficiency from exercise requires glycolysis to satisfy metabolic needs, while LDHB is expressed abundantly in cardiac muscle that is dependent upon aerobic metabolic pathways.

[3]Address correspondence to: Dr. E. Goldberg, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208; tel: 847 491-5416 ; fax: 847 467-1380 ; erv@northwestern.edu.

Our entrance into the field in its infancy was when we asked whether a single cell type, the spermatozoan, contained one or multiple forms of lactate dehydrogenase (Goldberg, 1963). That question began this journey with the discovery of LDH-X (now known as LDHC). This unique band corresponding to the homotetramer LDH-C_4 was only revealed in testis and spermatozoa and not in other tissues or cells. It was to our knowledge the first testis specific isozyme described (Blanco and Zinkham, 1963; Goldberg, 1963; Goldberg, 1964). Since that time, the question of why testes and sperm need this unique form of LDH remains to be discovered.

We learned that different forms of LDH are the product of 3 different genes: *Ldha*, *Ldhb*, and *Ldhc* which encode A, B and C subunits (Li, 1989). In the human and mouse genomes, *Ldha* and *Ldhc* genes are located in tandem on chromosomes 11 and 7 respectively (Edwards et al, 1989), and *Ldhb* gene on chromosomes 12 (human) and 6 (mouse) (Takeno and Li, 1989). In terms of gene evolution, the current model is that in vertebrate evolution, the original *Ldh* gene soon duplicated, giving rise to *Ldha* and *Ldhb* genes (Markert et al, 1975). The *Ldhc* gene then arose from a second independent gene duplication event, by duplication of the *Ldhb* gene in fish and columbid birds (Mannen et al, 1997;Zinkham et al, 1969), and by duplication of the *Ldha* gene during mammalian evolution (Li et al, 1983b;Li et al, 2002;Millan et al, 1987). Divergences in the structure, function and localization of the protein and in gene regulation must have conferred a genetic advantage since LDHC was conserved from its appearance throughout evolution. Finally, direct evidence of the importance of LDHC was demonstrated by the generation of an *Ldhc* knockout mouse model, but even though this model gave us some answers, it also raised new questions. LDHC still did not reveal all of its mysteries.

LDHC: CHARACTERISTICS OF THE PROTEIN

Structure and enzymatic characteristics of LDHC

The LDH isozymes differ in net charge which gave us the ability to electrophoretically distinguish them. In terms of activity, earlier studies showed that the kinetics of catalysis were different between the LDH isozymes (Goldberg, 1972). Mouse LDH-C_4 structure was characterized by crystallography (Goldberg, 1972; Musick and Rossmann, 1979) and complete sequences for LDH-C_4 became available (Pan et al, 1983). These structural studies confirmed that LDHC is homologous to the LDHA and LDHB subunits (72.5% and 75.3% identity with LDHA, and 64.5% and 69.8% identity with LDHB respectively in mouse and human). However, LDH-C_4 displays substitutions in amino acid sequence and some modifications in three-dimensional structure which gives LDH-C_4 unique structural (Hogrefe et al, 1987) and functional properties (Blanco et al, 1976; Li et al, 1983a). LDH-C_4 has high thermostability (Goldberg, 1972) and has a broader range of substrates; for example LDH-C_4 is able to metabolize α-hydroxyvalerate (Allen, 1961; Blanco et al, 1976; Goldberg, 1965). However the differences between LDH isozymes are not in the orders of magnitude sufficient to confer advantage to one of the three relative to germ cell metabolism (Goldberg, 1972).

Tissue localization

We showed the presence of LDHC protein in the testis using gel electrophoretic (Goldberg and Hawtrey, 1967) and immunohistochemical (Hintz and Goldberg, 1977) techniques (Figure 1). By using isolated fractions of mouse germ cells, the protein was detected first in preleptotene spermatocytes and was abundant in spermatids and spermatozoa (Li et al, 1989). LDHA also is present in pachytene spermatocytes, but the majority of LDH activity in male germ cells is from LDH-C_4 (Erickson et al, 1975a; Li et al, 1989; Odet et al, 2008),

and heterotetramers containing both A and C subunits are not detected in murine or human testes (Goldberg and Hawtrey, 1967; Li et al, 1989).

Our initial experiments suggested that LDHC was the only LDH isozyme present in spermatozoa (Goldberg, 1965). However, recent studies have shown that LDHA also is present in spermatozoa (Krisfalusi et al, 2006; Odet et al, 2008; Sleight et al, 2005). However, LDH-C_4 is responsible for more than 80% of the total LDH activity in mouse spermatozoa (Odet et al, 2008).

Originally, LDH-C_4 was considered specific to male germ cells (Blanco and Zinkham, 1963; Goldberg, 1963), while LDHB was described as the predominant LDH isozyme in oocytes (Roller et al, 1989). However, Coonrod et al. (2006) showed recently that LDHC protein can be detected by IHC in germinal-vesicle stage oocytes and fertilized eggs, and persists to the preimplantation blastocyst. However the enzymatic activity of LDH-C_4 was not detectable in egg extracts, presumably because of its low level, and *Ldhc* transcript levels are substantially lower in oocytes (102 per million transcripts) than in whole testis (2844 per million transcripts) (UniGene build #168, UniGene accession number for *Ldhc*: Mm.16563). The function(s) of LDH-C_4 during oogenesis, oocyte maturation, or early development is (are) still unclear. We do know that *Ldhc* null females are fertile (Odet et al, 2008).

Intracellular distribution of LDHC

Subcellular localization of a protein results in exposure to a specific microenvironment that might affect its function. Immunohistochemical studies have shown that LDHC is present in the cytosol of spermatocytes and spermatids, and in the principal piece of spermatozoa (Alvarez and Storey, 1984; Blanco et al, 1976; Burgos et al, 1995; Wheat and Goldberg, 1984) (Figure 2). In the sperm principal piece, LDHA but not LDHC was bound tightly to the fibrous sheath (Krisfalusi et al, 2006). This difference of intracellular localization between LDHA and LDHC might explain the absence of in vivo heterotetramers of A-C whereas different tetramer combinations were seen in vitro (Goldberg, 1965).

The relative hydrophobicity of LDHC (Millan et al, 1987) suggested that LDHC might be located close to the plasma membrane. Localization of LDHC in the cortical region in oocytes and preimplantation embryos (pronuclear zygotes to blastocysts) was observed (Coonrod et al, 2006). This is difficult to determine in sperm because of the small volume of the cytosol. However, by isolating different subcellular fractions of rabbit spermatozoa, Alvarez and Storey (1984) found 10 % of LDHC in the plasma membrane fraction. Consistent with this result, LDHC was also found at the surface of the spermatozoa (Beyler et al, 1985; Erickson et al, 1975a) possibly because of the high diffusibility of LDHC (Zinkham et al, 1964). However, the presence of extracellular LDHC might also have resulted from the release of LDHC during the isolation procedure from damaged cells.

Several studies have provided evidence that LDHC might be localized in the matrix of sperm type mitochondria (STM) (Alvarez and Storey, 1984; Burgos et al, 1995; Montamat et al, 1988). However, most of these data were based on indirect methods of detection and because of the high quantity of LDHC proteins present in spermatozoa, cross contamination between subcellular fractions is quite possible. Immunohistofluorescence studies with antibodies produced with peptides designated MC5-15 and MC211-220 (Beyler et al, 1985) detected strong signals in the principal piece of the spermatozoa (Duan and Goldberg, 2003; Goldberg, 1975; Odet et al, 2008), but only a weak signal in the midpiece region where the STM are localized (Figure 2). This weak signal was also found in *Ldhc* null sperm suggesting that it was only background (Odet, unpublished observation). However, LDHC localized in STM might have been lost due to the permeabilization and other treatments during preparation of the sperm smear. High resolution localization experiments using gold

immunolabeling, a specific antibody and spermatozoa from *Ldhc* null mice as negative controls are needed to resolve this controversy. However, it is worth mentioning that mitochondrial activity as measured by JC-1 staining and oxygen consumption (in medium with lactate as the only substrate) is normal in *Ldhc* null spermatozoa (Odet, unpublished observations); therefore, even if a small amount of LDHC is present in the matrix of STM, we were unable to detect any function for LDHC in the respiration process.

GENE EXPRESSION PATTERN AND REGULATION OF Ldhc GENE

Gene expression pattern in male germ cells

The level of *Ldhc* transcripts is higher in whole testis (2844 per million transcript; Unigene Mm.16563) than *Ldha* transcripts (584 per million; Mm.29324) and *Ldhb* transcripts (160 per million; Mm.9745). This is consistent with LDHC being the major LDH in germ cells and with germ cells being the major component of adult testis (>80%, mainly in the meiotic and postmeiotic phase). Like the protein, *Ldhc* transcripts were found in mouse testis only after 11 days post partum with the appearance of spermatocytes and then further increased with the appearance of spermatids (Alcivar et al, 1991; Goldberg, 1990; Thomas et al, 1990). This specific time course expression pattern of *Ldhc* was confirmed by microarray studies in the Griswold laboratory (Shima et al, 2004) (Figure 3). By using isolated testicular cell types they were also able to confirm that the *Ldhc* gene is expressed first in leptotene-zygotene spermatocytes with the highest level of *Ldhc* mRNA being present in spermatids (Figure 4). While *Ldhb* and *Ldha* transcripts were found in germ cells, they were at considerably lower levels than *Ldhc*. *Ldhb* was found mostly in spermatogonia.

Ldhc gene: transcriptional regulation

The human *LDHC* and murine *Ldhc* genes were cloned and sequenced (Cooker et al, 1993; Millan et al, 1987) and their promoter sequences defined (Zhou et al, 1994). Transcriptional analysis of these and other genes expressed in germ cells is compromised by the lack of a reliable cell culture system. Nevertheless, we have made progress in elucidating the regulation of *Ldhc* gene expression. Our approaches, construction of transgenic mice (Li et al, 1998; Markert et al, 1998), transfection of heterologous cell systems and injection of expression plasmids directly into seminiferous tubules via the rete testes have defined both cis and trans acting factors responsible for *Ldhc* transcription (Kroft et al, 2003; Tang and Goldberg, 2009; Tang et al, 2008). We confirmed and extended prior reports that transcription of the *Ldhc* gene begins with the onset of meiosis in male germ cells. The potential cis regulatory elements identified in studies using transgenic animals include a palindrome (-21 to +10) (PAL), GC box (-70 to -65) and CRE sites (-53 to -49, -39 to -35). We described a functional role for these sequences by expression of mutated transgenes in vivo. Our results (Tang et al, 2008) revealed for the first time that mutation of the GC box did not abolish promoter activity which remained testis-specific. Mutation of GC box or CRE sites resulted in a 73% and 74% reduction in promoter activity respectively in transient transfection of germ cells and in vivo by electroporation; the combination of GC box and CRE site mutations eliminated promoter activity whereas mutation of PAL had no effect. Therefore, we concluded that simultaneous occupancy of the GC box and CRE sites in the core promoter was necessary for full expression of *Ldhc* in the testis. Our working hypothesis proposes a partnership between the Sp and CREB factors mediated by an activator protein. We have been able to extend our studies to the human *Ldhc* promoter because it is activated in a malignant melanoma cell line. We determined that the murine and human promoters are only partially conserved since the latter sequence lacks a consensus TATA element upstream of the transcriptional start site. However, regulatory factors are similar for the murine and human *Ldhc* promoters (Tang and Goldberg, 2009). These findings are significant for developing an understanding of gene regulation in the

human testis. Human material would be difficult, if not impossible to obtain for such research. Overall, it appears that the role of the CRE site is essential for spermatocyte-specific gene expression.

Ldhc gene: non-transcriptional regulation

It has been demonstrated that the high level of *Ldhc* mRNA in germ cells is also the consequence of mRNA stability due to longer polyadenylation tails appearing with the meiotic divisions (Fujimoto et al, 1988). However, it seems that *Ldhc* mRNA stability is also species-specific and dependant on the presence of an AU-rich motif in the 3' untranslated region (3'UTR) (Salehi-Ashtiani and Goldberg, 1995). This information leads one to wonder if LDHC might be post-transcriptionally regulated by an inhibition of translation or an increase of mRNA stability by the new and exciting way of regulating transcript levels with non coding RNAs as microRNAs.

In the last decade, the importance of epigenetic regulation has emerged, especially during the highly regulated process of spermatogenesis (Zamudio et al, 2008). Genes frequently are hypomethylated in the testis (Oakes et al, 2007). In earlier studies, no differences were found in the methylation patterns of the *Ldhc* gene between somatic and germ cells (Alcivar et al, 1991) but the technique of restriction analysis of DNA methylation overlooked many potential methylation sites (Alcivar et al, 1991). Other studies demonstrated that the human promoter contains a mini-CpG island (Bonny and Goldberg, 1995), and that its methylation in non-expressing cells serves as the likely mechanism to suppress *Ldhc* gene activation (Tang and Goldberg, 2009).

FUNCTION(S) OF LDHC REVEALED (OR NOT) BY ITS ABSENCE

Male germ cell metabolism: why a need for special glycolytic isozymes?

LDHC was the first germ cell specific glycolytic isozyme discovered, but subsequent studies revealed that it was not the only one. Two other genes encoding isozymes for glyceraldehyde-3-phophate dehydrogenase type S (GAPDHS) (Welch et al, 2000; Welch et al, 1992) and phosphoglycerate kinase type 2 (PGK2) (Boer et al, 1987; McCarrey and Thomas, 1987) in mouse and human were found to be expressed only in spermatogenic cells. In addition, several glycolytic enzymes with unique properties were detected in male germ cells (reviewed in Miki, 2007) (Figure 5).

Several studies have shown that meiotic and post-meiotic male germ cells preferentially use lactate and pyruvate over glucose as an energy substrate (reviewed in Boussouar and Benahmed, 2004; Grootegoed et al, 1984; Jutte et al, 1982; Mita and Hall, 1982) suggesting that lactate oxidation by LDH isozymes has a significant role in energy metabolism during the middle and later stages of spermatogenesis. While these observations would fit with the abundance of LDHC in these stages of spermatogenesis, there is no direct evidence to support this contention. On the other hand, it has been shown that glycolysable substrates are essential for sperm motility (Mukai and Okuno, 2004; Williams and Ford, 2001), for the protein tyrosine phosphorylation indicative of capacitation (Travis et al, 2001; Urner et al, 2001; Urner and Sakkas, 2003; Williams and Ford, 2001) and for fertilization (Bone et al, 2000; Urner and Sakkas, 1996). This was proven when inactivation of the gene for the sperm-specific glycolysis pathway enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDHS) (Miki et al, 2004) dramatically reduced the level of ATP in sperm, caused severe defects in progressive sperm motility, and resulted in male infertility.

These data were consistent with our hypothesis that LDHC is essential for lactate metabolism during spermatogenesis, and for the conversion of pyruvate to lactate

accompanied by the generation of reduced NAD^+ (coenzyme essential for GAPDHS activity) in spermatozoa.

How to assess LDHC function(s)?

One approach to assess LDHC function in spermatozoa was the use of an LDH inhibitor. In bovine sperm, O'Flaherty et al. (2002) demonstrated that treatment with sodium oxamate (NaOx, pyruvate analog and LDH inhibitor) suppressed sperm capacitation. Similar results were obtained with mouse sperm (Duan and Goldberg, 2003). However, in addition to concerns about the specificity of the inhibitor, we found that it inhibited LDH activity efficiently in protein extracts, but not in intact sperm (Odet, unpublished observations). An alternative and ultimately successful approach was to assess LDHC function by targeted disruption of the *Ldhc* gene (Odet et al, 2008). Initial attempts were not successful, probably because of the abundance of repetitive sequence elements in the *Ldhc* gene (Olsson et al, 2003). However, when the *Ldhc* null mouse was generated, it proved to be an exciting model of male infertility and a unique biological resource that could lead to more complete understanding of the biochemical mechanisms underlying sperm function.

Ldhc null mice and spermatogenesis—Our first observation was that spermatogenesis appeared normal in *Ldhc* null mice. The testis histology looked unaffected and daily sperm production and sperm counts in the cauda epididymis were normal. To identify potential transcriptional modifications in *Ldhc* null testis, a microarray was performed in collaboration with Dr. Michael Griswold. However, preliminary results indicate that only *Ldhc* transcript levels were significantly different between WT and null testes by this method and by real-time PCR (Odet, unpublished observations). Although these results suggest that LDH-C_4 does not play an essential role in the maintenance of spermatogenesis, they do not rule out that the absence of LDH-C_4 has a subtle effect on spermatogenesis that subsequently compromises sperm function.

Ldhc null male mice and fertilization—Although spermatogenesis appeared normal, the fertility of *Ldhc* null males was severely compromised. Our initial study was performed with animals from the F3 and F5 back-cross generations on the C57Bl/6N background (predicted 12.5% and 3.13% residual 129svEv background, respectively). From a total of 11 different *Ldhc* null males mated with more than 44 WT females over a 2 to 4 months period, only 2 litters of 1 and 3 pups were recovered (Odet et al, 2008).

Other studies demonstrated that this extreme subfertility was a consequence of a defect(s) in sperm function. Motility of *Ldhc* null sperm was impaired and their progressive motility decreased over time suggesting that *Ldhc* null sperm are unable to swim through the female tract (Odet et al, 2008). Moreover, even if *Ldhc* null sperm were able to reach the eggs, IVF experiments indicated that they were unable to fertilize oocytes. *Ldhc* null sperm did not undergo the protein tyrosine phosphorylation changes characteristic of capacitation and were unable to develop hyperactivated motility, processes essential for fertilization. When the zona pellucida was removed, *Ldhc* null sperm fertilized 29% of eggs. This was a significantly lower percentage of fertilization than occurred with sperm from heterozygote (HET) mice suggesting that, in addition to a defect in the mechanical process of oocyte penetration through the zona pellucida, there is also a defect in fusion to oocytes by *Ldhc* null sperm.

Ldhc null mice and sperm metabolism—While it was clear that sperm defects were responsible for the infertility, the causes of the defects remained to be determined. A high rate of ATP production in sperm is known to be essential for maintaining a high level of motility for a prolonged period of time, and to induce sperm capacitation and hyperactivity.

We hypothesized that lack of LDHC would induce a defect in ATP production by inhibition of glycolysis, the principal source of ATP in most mammalian sperm. Consistent with this hypothesis, we found rapid decreases in progressive motility and ATP levels over time in *Ldhc* null sperm than in WT sperm (Odet et al, 2008). By following the transformation of ^{13}C-labelled glucose using NMR spectroscopy, we found that unlike WT sperm, *Ldhc* null sperm were unable to consume glucose (Odet, unpublished observations). However, by using direct (consumption of ^{13}C-pyruvate, NMR spectroscopy) and indirect methods (amount and rate of lactate production from pyruvate) we also observed an unexpected result: *Ldhc* null sperm were able to convert pyruvate into lactate with the same rapid kinetics as WT sperm (Odet, unpublished observations). Because LDHA has been found in association with the isolated fibrous sheath, these results strongly suggest that LDHA is responsible for some or most of the LDH activity in *Ldhc* null sperm.

It is worth mentioning that sperm from mice heterozygous for the *Ldhc* mutation were fertile, whereas *Ldhc* transcript levels were reduced by 40% in the testes of these mice, and global LDH activity was reduced by 19.1% in testis and 24.7% in sperm. These observations indicate that sperm contain substantially more LDH-C_4 than is required to maintain normal fertility.

CONCLUSION

Finding that the function of sperm from *Ldhc* null mice is severely compromised confirms the importance of this isozyme and strongly justifies further study of the role it plays in conferring or maintaining sperm fertilizing capacity. As has been noted previously, these studies are of translational relevance to problems of male infertility (Gavella and Cvitkovic, 1985; Orlando et al, 1988) and to the development of a male contraceptive (Erickson et al, 1975b; Goldberg, 1973; Goldberg et al, 2001; Mahi-Brown et al, 1990; O'Hern et al, 1995).

Therefore, we still must ask why sperm contain such a large quantity of LDHC. What could be the function of LDHC if LDHA alone can provide the terminal reaction of glycolysis? Many questions about LDHC remain to be elucidated.

Acknowledgments

This research was supported in part by NIH HD05863 (EG) and in part by the Intramural Research Program of the NIH, National Institutes of Environmental Health Sciences (EME).

References

Alcivar AA, Trasler JM, Hake LE, Salehi-Ashtiani K, Goldberg E, Hecht NB. DNA methylation and expression of the genes coding for lactate dehydrogenases A and C during rodent spermatogenesis. Biol Reprod 1991;44:527–535. [PubMed: 2015369]

Allen JM. Multiple forms of lactic dehydrogenase in tissues of the mouse: their specificity, cellular localization, and response to altered physiological conditions. Ann N Y Acad Sci 1961;94:937–951. [PubMed: 13860552]

Alvarez JG, Storey BT. Assessment of cell damage caused by spontaneous lipid peroxidation in rabbit spermatozoa. Biol Reprod 1984;30:323–331. [PubMed: 6231058]

Beyler SA, Wheat TE, Goldberg E. Binding of antibodies against antigenic domains of murine lactate dehydrogenase-C4 to human and mouse spermatozoa. Biol Reprod 1985;32:1201–1210. [PubMed: 2410040]

Blanco A, Burgos C, Gerez de Burgos NM, Montamat EE. Properties of the testicular lactate dehydrogenase isoenzyme. The Biochemical journal 1976;153:165–172. [PubMed: 1275882]

Blanco A, Zinkham WH. Lactate Dehydrogenases in Human Testes. Science 1963;139:601–602. [PubMed: 17788300]

Boer PH, Adra CN, Lau YF, McBurney MW. The testis-specific phosphoglycerate kinase gene pgk-2 is a recruited retroposon. Mol Cell Biol 1987;7:3107–3112. [PubMed: 2823118]

Bone W, Jones NG, Kamp G, Yeung CH, Cooper TG. Effect of ornidazole on fertility of male rats: inhibition of a glycolysis-related motility pattern and zona binding required for fertilization in vitro. J Reprod Fertil 2000;118:127–135. [PubMed: 10793634]

Bonny C, Goldberg E. The CpG-rich promoter of human LDH-C is differentially methylated in expressing and nonexpressing tissues. Developmental genetics 1995;16:210–217. [PubMed: 7736669]

Boussouar F, Benahmed M. Lactate and energy metabolism in male germ cells. Trends Endocrinol Metab 2004;15:345–350. [PubMed: 15350607]

Burgos C, Maldonado C, Gerez de Burgos NM, Aoki A, Blanco A. Intracellular localization of the testicular and sperm-specific lactate dehydrogenase isozyme C4 in mice. Biol Reprod 1995;53:84–92. [PubMed: 7669861]

Cooker LA, Brooke CD, Kumari M, Hofmann MC, Millan JL, Goldberg E. Genomic structure and promoter activity of the human testis lactate dehydrogenase gene. Biol Reprod 1993;48:1309–1319. [PubMed: 8318584]

Coonrod S, Vitale A, Duan C, Bristol-Gould S, Herr J, Goldberg E. Testis-specific lactate dehydrogenase (LDH-C4; Ldh3) in murine oocytes and preimplantation embryos. J Androl 2006;27:502–509. [PubMed: 16582413]

Dewey MM, Conklin JL. Starch gel electrophoresis of lactic dehydrogenase from rat kidney. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine 1960;105:492–494.

Duan C, Goldberg E. Inhibition of lactate dehydrogenase C4 (LDH-C4) blocks capacitation of mouse sperm in vitro. Cytogenet Genome Res 2003;103:352–359. [PubMed: 15051959]

Edwards Y, West L, Van Heyningen V, Cowell J, Goldberg E. Regional localization of the sperm-specific lactate dehydrogenase, LDHC, gene on human chromosome 11. Annals of human genetics 1989;53:215–219. [PubMed: 2596827]

Erickson RP, Friend DS, Tennenbaum D. Localization of lactate dehydrogenase-X on the surfaces of mouse spermatozoa. Experimental cell research 1975a;91:1–5. [PubMed: 1093856]

Erickson RP, Stites DP, Spielmann H. Effects on fertility of immunization against lactate dehydrogenase X in female mice. Contraception 1975b;12:333–338. [PubMed: 1172463]

Everse J, Kaplan NO. Lactate dehydrogenases: structure and function. Adv Enzymol Relat Areas Mol Biol 1973;37:61–133. [PubMed: 4144036]

Fujimoto H, Erickson RP, Tone S. Changes in polyadenylation of lactate dehydrogenase-X mRNA during spermatogenesis in mice. Mol Reprod Dev 1988;1:27–34. [PubMed: 2908441]

Gavella M, Cvitkovic P. Semen LDH-X deficiency and male infertility. Arch Androl 1985;15:173–176. [PubMed: 3833074]

Goldberg E. Lactic and Malic Dehydrogenases in Human Spermatozoa. Science 1963;139:602–603. [PubMed: 17788301]

Goldberg E. Lactate Dehydrogenases and Malate Dehydrogenases in Sperm: Studied by Polyacrylamide Gel Electrophoresis. Ann N Y Acad Sci 1964;121:560–570. [PubMed: 14240552]

Goldberg E. Lactate Dehydrogenases in Spermatozoa: Subunit Interactions in Vitro. Arch Biochem Biophys 1965;109:134–141. [PubMed: 14281937]

Goldberg E. Amino acid composition and properties of crystalline lactate dehydrogenase X from mouse testes. J Biol Chem 1972;247:2044–2048. [PubMed: 4622741]

Goldberg E. Infertility in female rabbits immunized with lactate dehydrogenase X. Science 1973;181:458–459. [PubMed: 4718114]

Goldberg E. Lactate dehydrogenase-X from mouse testes and spermatozoa. Methods Enzymol 1975;41:318–323. [PubMed: 1128270]

Goldberg E. Developmental expression of lactate dehydrogenase isozymes during spermatogenesis. Prog Clin Biol Res 1990;344:49–52. [PubMed: 2203052]

Goldberg E, Hawtrey C. The ontogeny of sperm specific lactate dehydrogenase in mice. J Exp Zool 1967;164:309–316. [PubMed: 6068185]

Goldberg E, VandeBerg JL, Mahony MC, Doncel GF. Immune response of male baboons to testis-specific LDH-C(4). Contraception 2001;64:93–98. [PubMed: 11704085]

Grootegoed JA, Jansen R, Van der Molen HJ. The role of glucose, pyruvate and lactate in ATP production by rat spermatocytes and spermatids. Biochim Biophys Acta 1984;767:248–256. [PubMed: 6498180]

Hintz M, Goldberg E. Immunohistochemical localization of LDH-x during spermatogenesis in mouse testes. Dev Biol 1977;57:375–384. [PubMed: 326599]

Hogrefe HH, Griffith JP, Rossmann MG, Goldberg E. Characterization of the antigenic sites on the refined 3-A resolution structure of mouse testicular lactate dehydrogenase C4. J Biol Chem 1987;262:13155–13162. [PubMed: 2443489]

Jutte NH, Jansen R, Grootegoed JA, Rommerts FF, Clausen OP, van der Molen HJ. Regulation of survival of rat pachytene spermatocytes by lactate supply from Sertoli cells. J Reprod Fertil 1982;65:431–438. [PubMed: 6808129]

Krisfalusi M, Miki K, Magyar PL, O'Brien DA. Multiple glycolytic enzymes are tightly bound to the fibrous sheath of mouse spermatozoa. Biol Reprod 2006;75:270–278. [PubMed: 16687649]

Kroft TL, Li S, Doglio L, Goldberg E. A transgenic analysis of mouse lactate dehydrogenase C promoter activity in the testis. J Androl 2003;24:843–852. [PubMed: 14581510]

Li S, Zhou W, Doglio L, Goldberg E. Transgenic mice demonstrate a testis-specific promoter for lactate dehydrogenase, LDHC. J Biol Chem 1998;273:31191–31194. [PubMed: 9813024]

Li SS. Lactate dehydrogenase isoenzymes A (muscle), B (heart) and C (testis) of mammals and the genes coding for these enzymes. Biochemical Society transactions 1989;17:304–307. [PubMed: 2753209]

Li SS, Feldmann RJ, Okabe M, Pan YC. Molecular features and immunological properties of lactate dehydrogenase C4 isozymes from mouse and rat testes. J Biol Chem 1983a;258:7017–7028. [PubMed: 6189833]

Li SS, Fitch WM, Pan YC, Sharief FS. Evolutionary relationships of vertebrate lactate dehydrogenase isozymes A4 (muscle), B4 (heart), and C4 (testis). J Biol Chem 1983b;258:7029–7032. [PubMed: 6853510]

Li SS, O'Brien DA, Hou EW, Versola J, Rockett DL, Eddy EM. Differential activity and synthesis of lactate dehydrogenase isozymes A (muscle), B (heart), and C (testis) in mouse spermatogenic cells. Biol Reprod 1989;40:173–180. [PubMed: 2923949]

Li YJ, Tsoi SC, Mannen H, Shoei-lung Li S. Phylogenetic analysis of vertebrate lactate dehydrogenase (LDH) multigene families. Journal of molecular evolution 2002;54:614–624. [PubMed: 11965434]

Mahi-Brown CA, VandeVoort CA, McGuinness RP, Overstreet JW, O'Hern P, Goldberg E. Immunization of male but not female mice with the sperm-specific isozyme of lactate dehydrogenase (LDH-C4) impairs fertilization in vivo. Am J Reprod Immunol 1990;24:1–8. [PubMed: 2285453]

Mannen H, Tsoi SC, Krushkal JS, Li WH, Li SS. The cDNA cloning and molecular evolution of reptile and pigeon lactate dehydrogenase isozymes. Molecular biology and evolution 1997;14:1081–1087. [PubMed: 9364765]

Markert CL, Amet TM, Goldberg E. Human testis-specific lactate dehydrogenase-C promoter drives overexpression of mouse lactate dehydrogenase-1 cDNA in testes of transgenic mice. J Exp Zool 1998;282:171–178. [PubMed: 9723174]

Markert CL, Appella E. Physicochemical nature of isozvmes. Ann N Y Acad Sci 1961;94:678–690. [PubMed: 14469961]

Markert CL, Shaklee JB, Whitt GS. Evolution of a gene. Multiple genes for LDH isozymes provide a model of the evolution of gene structure, function and regulation. Science 1975;189:102–114. [PubMed: 1138367]

McCarrey JR, Thomas K. Human testis-specific PGK gene lacks introns and possesses characteristics of a processed gene. Nature 1987;326:501–505. [PubMed: 3453121]

Miki K. Energy metabolism and sperm function. Soc Reprod Fertil Suppl 2007;65:309–325. [PubMed: 17644971]

Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, Strader LF, Perreault SD, Eddy EM, O'Brien DA. Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is

required for sperm motility and male fertility. Proc Natl Acad Sci U S A 2004;101:16501–16506. [PubMed: 15546993]

Millan JL, Driscoll CE, LeVan KM, Goldberg E. Epitopes of human testis-specific lactate dehydrogenase deduced from a cDNA sequence. Proc Natl Acad Sci U S A 1987;84:5311–5315. [PubMed: 2440048]

Mita M, Hall PF. Metabolism of round spermatids from rats: lactate as the preferred substrate. Biol Reprod 1982;26:445–455. [PubMed: 7082719]

Montamat EE, Vermouth NT, Blanco A. Subcellular localization of branched-chain amino acid aminotransferase and lactate dehydrogenase C4 in rat and mouse spermatozoa. The Biochemical journal 1988;255:1053–1056. [PubMed: 3214422]

Mukai C, Okuno M. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. Biol Reprod 2004;71:540–547. [PubMed: 15084484]

Musick WD, Rossmann MG. The structure of mouse testicular lactate dehydrogenase isoenzyme C4 at 2.9 A resolution. J Biol Chem 1979;254:7611–7620. [PubMed: 468772]

O'Hern PA, Bambra CS, Isahakia M, Goldberg E. Reversible contraception in female baboons immunized with a synthetic epitope of sperm-specific lactate dehydrogenase. Biol Reprod 1995;52:331–339. [PubMed: 7536050]

Oakes CC, La Salle S, Smiraglia DJ, Robaire B, Trasler JM. A unique configuration of genome-wide DNA methylation patterns in the testis. Proc Natl Acad Sci U S A 2007;104:228–233. [PubMed: 17190809]

Odet F, Duan C, Willis WD, Goulding EH, Kung A, Eddy EM, Goldberg E. Expression of the Gene for Mouse Lactate Dehydrogenase C (Ldhc) Is Required for Male Fertility. Biol Reprod 2008;79(1):26–34. [PubMed: 18367675]

Olsson PG, Tsujioka H, Narisawa S, Goldberg E, Millan JL. Abundance of repetitive sequence elements in the mouse testis-specific lactate dehydrogenase-C gene. J Androl 2003;24:918–920. [PubMed: 14581519]

Orlando C, Casano R, Caldini AL, Forti G, Barni T, Bonfanti L, Serio M. Measurement of seminal LDH-X and transferrin in normal and infertile men. J Androl 1988;9:220–223. [PubMed: 3403363]

Pan YC, Sharief FS, Okabe M, Huang S, Li SS. Amino acid sequence studies on lactate dehydrogenase C4 isozymes from mouse and rat testes. J Biol Chem 1983;258:7005–7016. [PubMed: 6343385]

Roller RJ, Kinloch RA, Hiraoka BY, Li SS, Wassarman PM. Gene expression during mammalian oogenesis and early embryogenesis: quantification of three messenger RNAs abundant in fully grown mouse oocytes. Development 1989;106:251–261. [PubMed: 2591314]

Salehi-Ashtiani K, Goldberg E. Posttranscriptional regulation of primate Ldhc mRNA by its AUUUA-like elements. Mol Endocrinol 1995;9:1782–1790. [PubMed: 8614414]

Shima JE, McLean DJ, McCarrey JR, Griswold MD. The murine testicular transcriptome: characterizing gene expression in the testis during the progression of spermatogenesis. Biol Reprod 2004;71:319–330. [PubMed: 15028632]

Sleight SB, Miranda PV, Plaskett NW, Maier B, Lysiak J, Scrable H, Herr JC, Visconti PE. Isolation and proteomic analysis of mouse sperm detergent-resistant membrane fractions: evidence for dissociation of lipid rafts during capacitation. Biol Reprod 2005;73:721–729. [PubMed: 15917346]

Smithies O. Zone electrophoresis in starch gels and its application to studies of serum proteins. Advances in protein chemistry 1959;14:65–113. [PubMed: 13832188]

Takeno T, Li SS. Structure of the human lactate dehydrogenase B gene. The Biochemical journal 1989;257:921–924. [PubMed: 2930497]

Tang H, Goldberg E. Homo sapiens lactate dehydrogenase c (Ldhc) gene expression in cancer cells is regulated by transcription factor Sp1, CREB, and CpG island methylation. J Androl 2009;30:157–167. [PubMed: 18930904]

Tang H, Kung A, Goldberg E. Regulation of murine lactate dehydrogenase C (Ldhc) gene expression. Biol Reprod 2008;78:455–461. [PubMed: 18057313]

Thomas K, Del Mazo J, Eversole P, Bellve A, Hiraoka Y, Li SS, Simon M. Developmental regulation of expression of the lactate dehydrogenase (LDH) multigene family during mouse spermatogenesis. Development 1990;109:483–493. [PubMed: 2401207]

Travis AJ, Jorgez CJ, Merdiushev T, Jones BH, Dess DM, Diaz-Cueto L, Storey BT, Kopf GS, Moss SB. Functional relationships between capacitation-dependent cell signaling and compartmentalized metabolic pathways in murine spermatozoa. J Biol Chem 2001;276:7630–7636. [PubMed: 11115497]

Urner F, Leppens-Luisier G, Sakkas D. Protein tyrosine phosphorylation in sperm during gamete interaction in the mouse: the influence of glucose. Biol Reprod 2001;64:1350–1357. [PubMed: 11319138]

Urner F, Sakkas D. Glucose participates in sperm-oocyte fusion in the mouse. Biol Reprod 1996;55:917–922. [PubMed: 8879509]

Urner F, Sakkas D. Protein phosphorylation in mammalian spermatozoa. Reproduction 2003;125:17–26. [PubMed: 12622692]

Welch JE, Brown PL, O'Brien DA, Magyar PL, Bunch DO, Mori C, Eddy EM. Human glyceraldehyde 3-phosphate dehydrogenase-2 gene is expressed specifically in spermatogenic cells. J Androl 2000;21:328–338. [PubMed: 10714828]

Welch JE, Schatte EC, O'Brien DA, Eddy EM. Expression of a glyceraldehyde 3-phosphate dehydrogenase gene specific to mouse spermatogenic cells. Biol Reprod 1992;46:869–878. [PubMed: 1375514]

Wheat TE, Goldberg E. Immunochemical dissection of the testes-specific isozyme lactate dehydrogenase C4. Ann N Y Acad Sci 1984;438:156–170. [PubMed: 6085454]

Williams AC, Ford WC. The role of glucose in supporting motility and capacitation in human spermatozoa. J Androl 2001;22:680–695. [PubMed: 11451366]

Zamudio NM, Chong S, O'Bryan MK. Epigenetic regulation in male germ cells. Reproduction 2008;136:131–146. [PubMed: 18515312]

Zhou W, Xu J, Goldberg E. A 60-bp core promoter sequence of murine lactate dehydrogenase C is sufficient to direct testis-specific transcription in vitro. Biol Reprod 1994;51:425–432. [PubMed: 7803614]

Zinkham WH, Blanco A, Clowry LJ Jr. An Unusual Isozyme of Lactate Dehydrogenase in Mature Testes: Localization, Ontogeny, and Kinetic Properties. Ann N Y Acad Sci 1964;121:571–588. [PubMed: 14240553]

Zinkham WH, Isensee H, Renwick JH. Linkage of lactate dehydrogenase B and C loci in pigeons. Science 1969;164:185–187. [PubMed: 5774192]

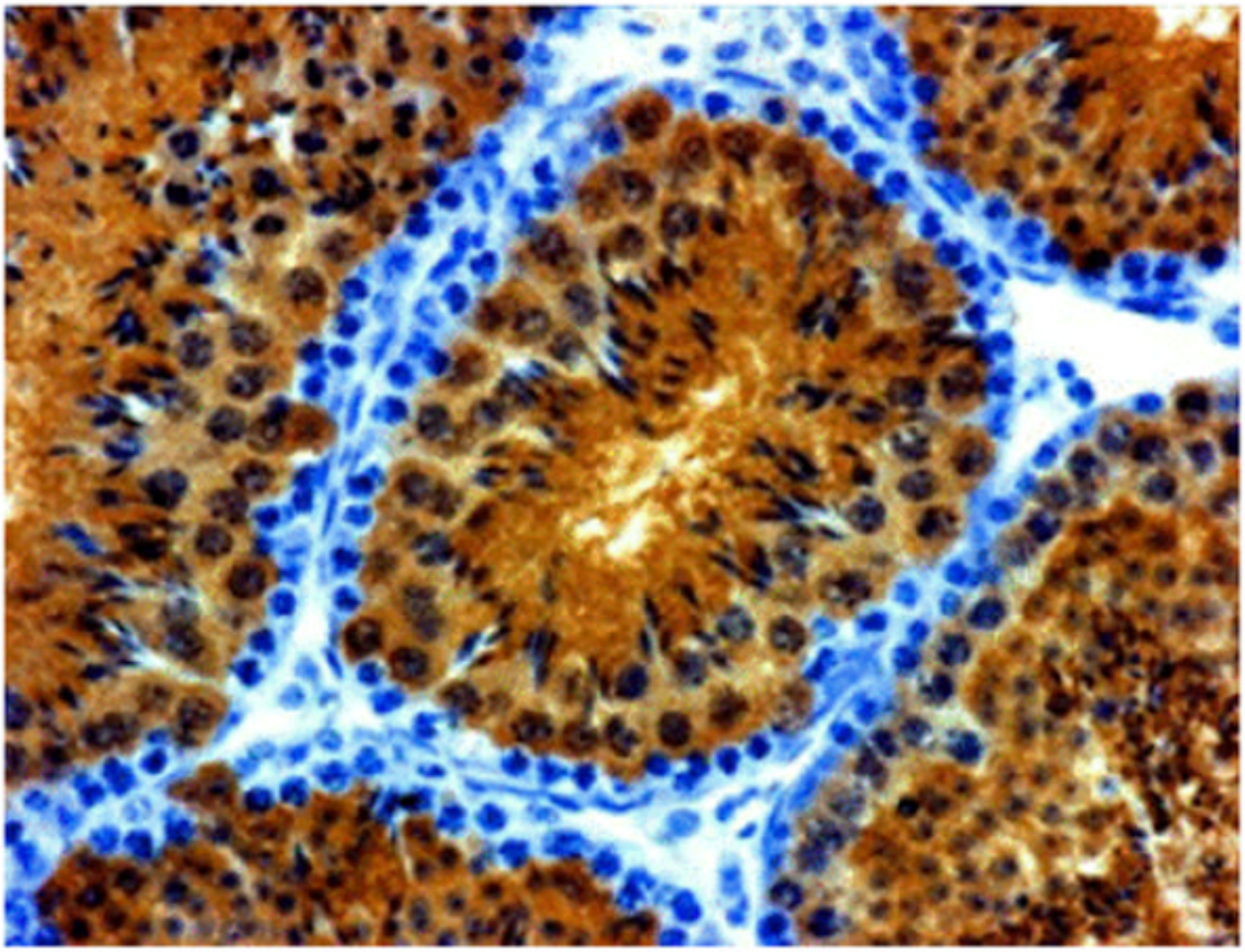

Figure 1.
Immunohistochemical staining of mouse testis section with antibody to LDHC and a Histostain Plus Rabbit Primary (DAB) kit (Zymed Laboratories, San Francisco, CA). The brown reaction product is visible only in pachytene and later stages of spermatogenesis.

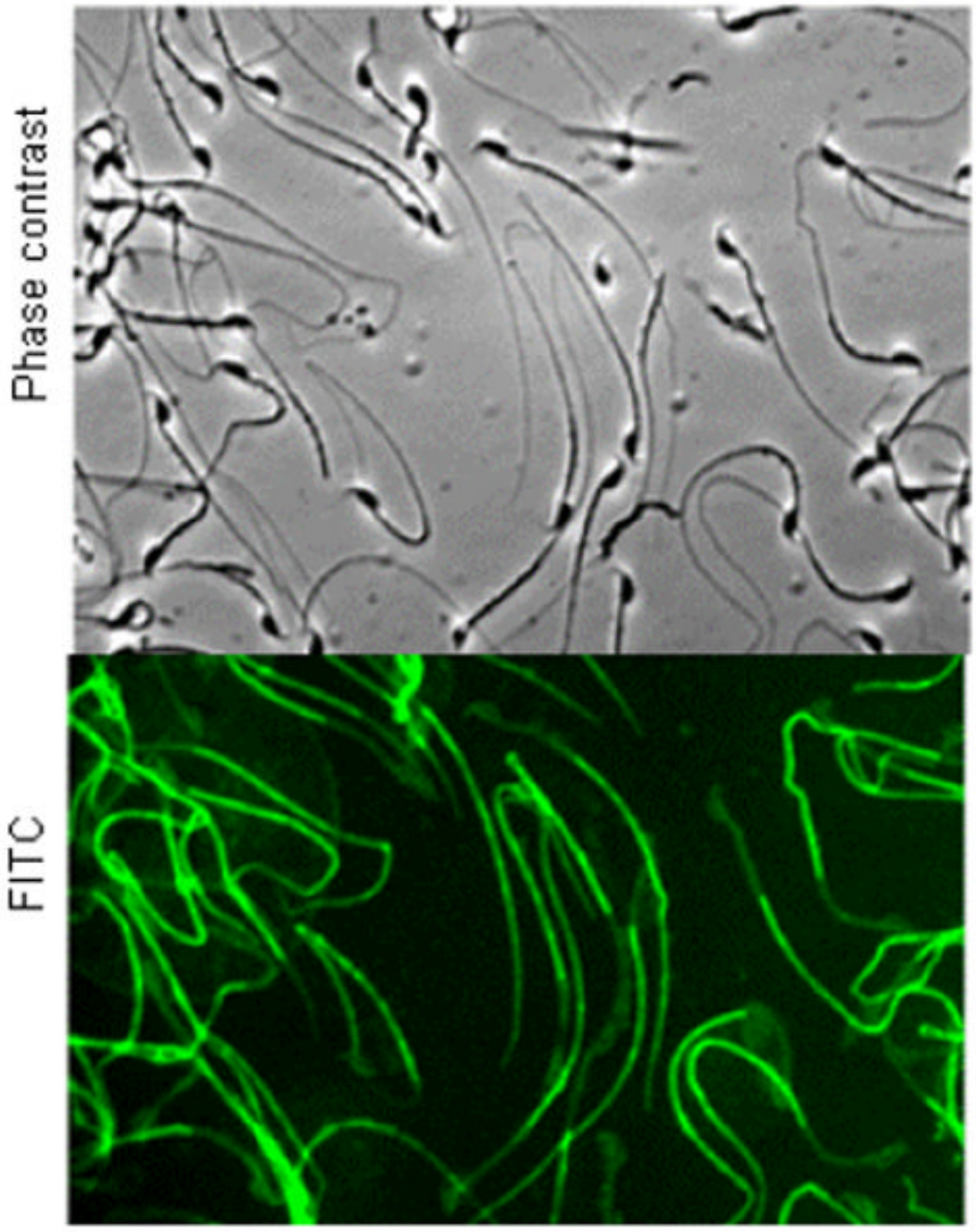

Figure 2.
Immunofluorescent localization of LDHC in mouse sperm. The protein is most abundant in the sperm principle piece; however there is a weaker signal in the mid-piece and on the head. Sperm were dried on a microscope slide, incubated with primary antibody to LDHC followed by fluorescein labeled secondary antibody.

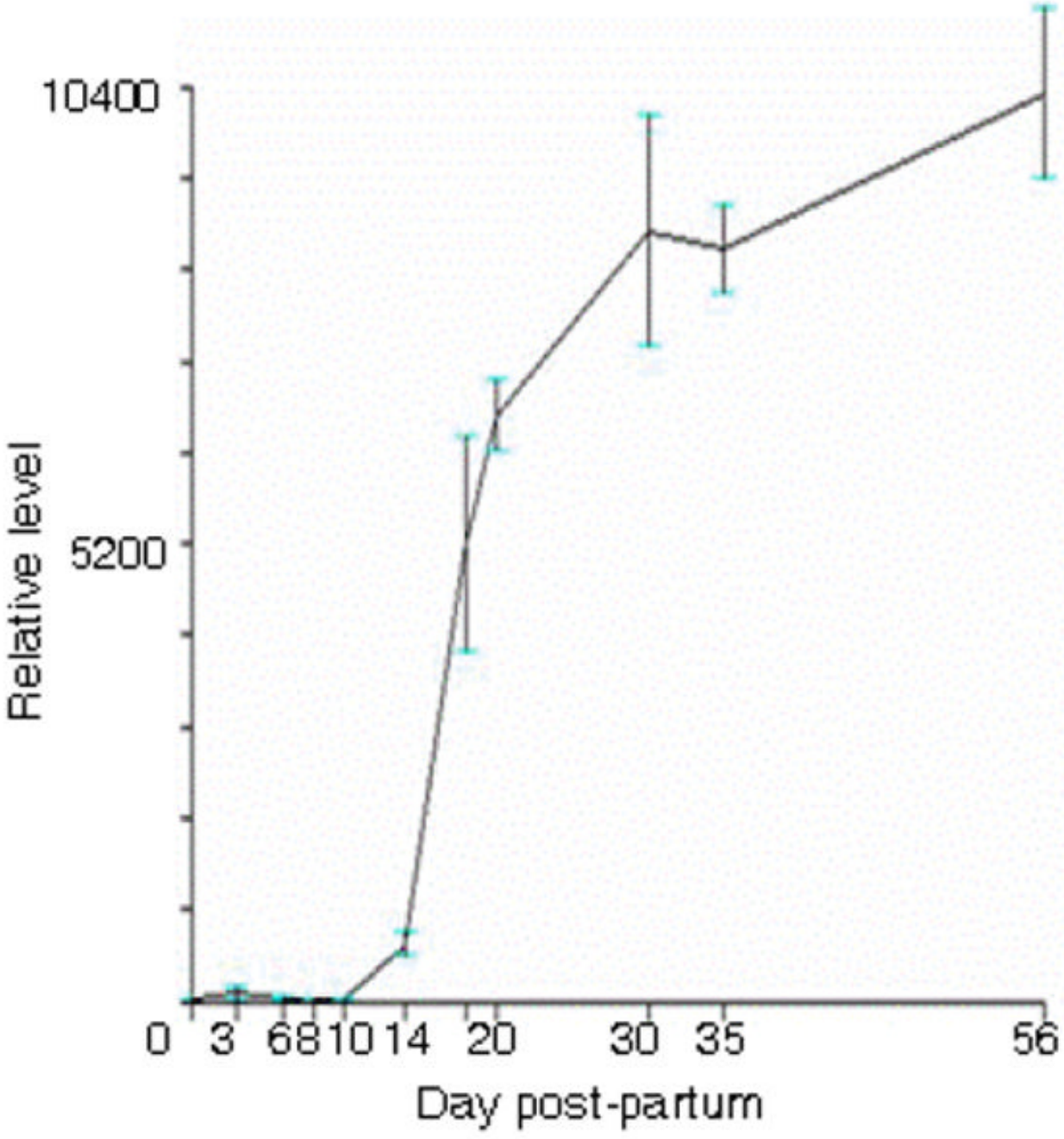

Figure 3.
Developmental expression for Ldhc determined in micro-array experiments (see Shima et al, 2004). Unpublished data courtesy of M.Griswold.

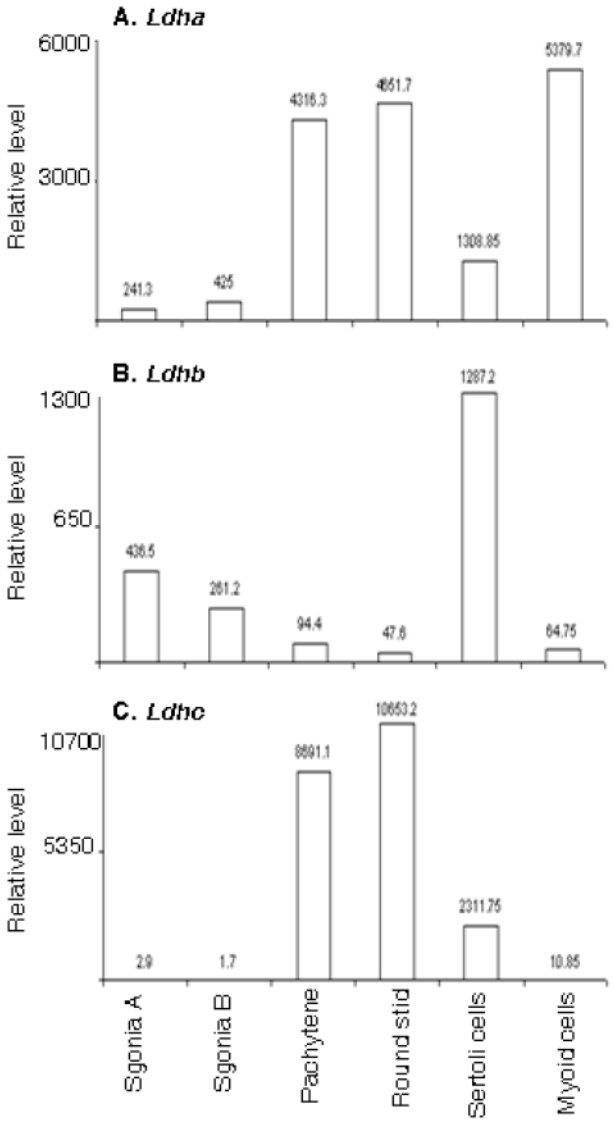

Figure 4.

Distribution of lactate dehydrogenase a (A), b (B) and c (C) mRNAs in different cellular fraction of mouse testis: spermatogonia typa A (SgoniaA), spermatogonia type B (SgoniaB), pachytene spermatocyte (pachytene), round spermatid (round stid), Sertoli cells, and myoid cells. Unpublished data courtesy of M.Griswold.

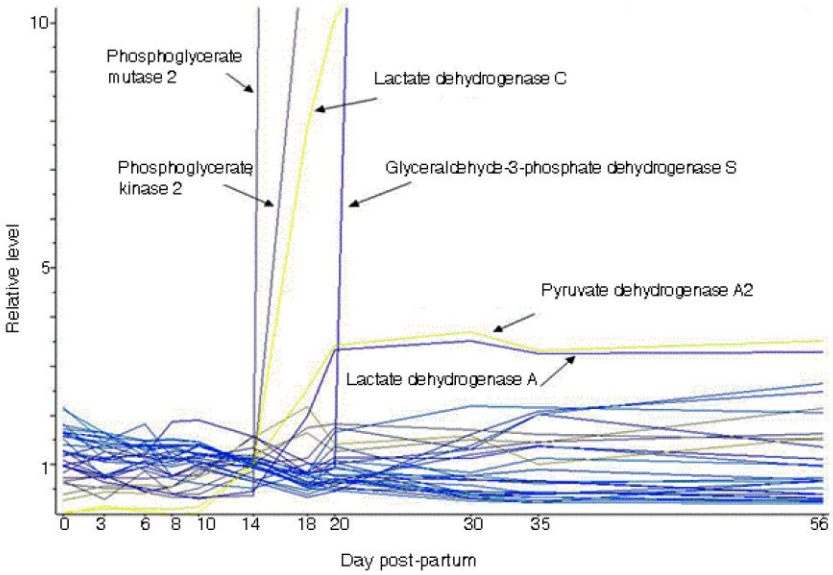

Figure 5.
Developmental expression in mouse testis of select genes for glycolytic enzymes. Unpublished data courtesy of M.Griswold