Published in final edited form as:

J Urol. 2012 October ; 188(4 Suppl): 1411–1416. doi:10.1016/j.juro.2012.06.033.

Reduced Expression of Androgen Receptor and Myosin Heavy Chain mRNA in Cremaster Muscle of Boys with Nonsyndromic Cryptorchidism

Julia Spencer Barthold, Yanping Wang, Anita Reilly, Alan Robbins, T. Ernesto Figueroa, Ahmad BaniHani, Jennifer Hagerty, and Robert E. Akins

Surgery/Urology and Nemours Biomedical Research, Al duPont Hospital for Children, Wilmington, DE

Abstract

Purpose—To better define developmental mechanisms of nonsyndromic cryptorchidism (NSC), we measured expression of hormone receptor and muscle type-specific mRNAs in target tissues of boys with and without NSC.

Materials and Methods—Prospectively collected cremaster muscle and/or hernia sac tissues from boys with congenital (n=79) or acquired (n=66) NSC and hernia/hydrocele (controls, n=84) were analyzed for hormone receptor (*RXFP2*, *AR*, *ESR1*, *ESR2*) and myosin heavy chain-specific (*MYH1*, *MYH2*, *MYH7*) mRNA expression using real time RT-PCR. Log-transformed mRNA, phenotype and feeding history data were statistically analyzed using Pearson's correlation, ANOVA and 2-sample T tests.

Results—*AR* mRNA expression was higher in cremaster than sac, and significantly lower in both congenital and acquired NSC relative to controls (p<0.01). Type 1 (slow/cardiac) *MYH7* mRNA expression was also significantly reduced in both NSC groups (p = 0.002) while a reduction in type 2 (fast) *MYH2* expression was more modest and significant only for the congenital group (p<0.05). Cremasteric *MYH7* and *AR* levels were strongly correlated ($r^2 = .751$, p<0.001). *MYH7* and *ESR1* mRNA levels were higher and lower, respectively, in soy formula-fed boys with NSC. Expression of other genes was not measurable.

Conclusions—Our data suggest that boys with congenital and acquired NSC differentially express *AR* and slow twitch-specific *MYH7* mRNA in cremaster and that *MYH7* expression is correlated with *AR* levels and soy formula use. These differences in gene expression may reflect aberrant hormonal signaling and/or innervation during development with the potential for secondary functional effects and failed testicular descent.

Keywords

Cryptorchidism; androgen; estrogen; myosin; cremaster

Introduction

Isolated failure of testicular descent to scrotal position, or nonsyndromic cryptorchidism (NSC), occurs in 2–3% of boys and is associated with long-term risks of subfertility and testicular malignancy.¹ NSC is associated with a constellation of reproductive tract and developmental abnormalities that may reflect damaging genetic and/or environmental influences. Epididymal defects, intrauterine growth retardation, subtle abnormalities of the hypothalamic-pituitary-gonadal axis, and impaired spermatogonial development may occur in affected infants with NSC, and these varied phenotypes may have a common underlying etiology. Although the etiology of NSC in man is unknown, it is hypothesized to be

multifactorial and most likely due to altered signaling of insulin-like 3/relaxin/insulin-like family peptide receptor 2 (INSL3/RXFP2) and/or androgen receptor (AR) pathways, which are essential for gubernacular development.² INSL3/RXFP2 signaling is essential for testicular descent while AR plays a more general role in genital development, but specific mutations of these genes are uncommon in NSC.³

The mammalian gubernaculum comprises mesenchymal cells and an intrinsic (rodent) or extrinsic (human) cremaster muscle layer. The cell biology of hormone signaling within the gubernaculum remains incompletely defined. *In vitro* studies suggest that INSL3 and androgen stimulate gubernacular proliferation and that their effects may be additive,^{4,5} but the target cell(s) for hormonal signaling in the gubernaculum have not been specifically identified. Interestingly, *Rxfp2* mRNA expression is diffuse throughout the fetal rat gubernaculum on embryonic (E) day 14,⁶ but immunostaining for INSL3 in the gubernaculum, which presumably localizes cell surface RXFP2, is limited to the muscle layer by E18⁷ in mice suggesting that muscle may be the target of INSL3/RXFP2 signaling during a critical phase of testicular descent. AR and ER α proteins are also expressed in muscle, and AR has been found in the central mesenchyme of the rodent gubernaculum, possibly in fibroblasts or myofibroblasts, at E14.5–16.5 (mice) and E21.5 (rats).^{8,9} Exposure to the antiandrogen flutamide during a critical window of gestation (E16–19)¹⁰ causes cryptorchidism in rats, but is accompanied by minimal inhibition of fetal gubernacular size,^{11,12} suggesting that the antiandrogen effect may not be associated with gubernacular growth per se. In addition, it was recently shown that flutamide reduces *Ins3* mRNA expression in fetal rats,¹³ suggesting that a mechanism other than AR blockade may contribute to flutamide-induced cryptorchidism. Evidence also exists for interactions between *Ins3* and androgen signaling,¹⁴ but details of how the complex interplay between *Ins3* and androgen signaling determine testicular position have not been worked out.

An emerging theme in deciphering mechanisms of testicular descent involves the role of muscle. Cryptorchidism occurs commonly in many syndromes associated with abnormal neuromuscular development¹⁵ supporting a role for muscle activity in the process of testicular descent. The cremaster muscle expresses both *Rxfp2* and *Ar* in fetal mice and consequently may represent a key target of critical hormone activities. Androgens stimulate myoblast differentiation and muscle hypertrophy, and regulate fiber type in skeletal muscle.¹⁶ Accordingly, the present studies test the hypothesis that altered expression of hormone receptor and muscle type-specific mRNAs are present in target tissues of boys with NSC, potentially reflecting aberrant hormone-dependent signaling during development.

Materials and Methods

Patients

Clinical data and tissue samples were collected from boys recruited prospectively in IRB-approved protocols with informed consent. We identified prepubertal participants attending a pediatric urology clinic scheduled for surgery to correct cryptorchidism (cases, n=145) or inguinal hernia/communicating hydrocele (controls, n=84). Exclusion criteria included reported gestational age <36 weeks, other genital anomalies including hypospadias or micropenis; major abdominal wall defect or any defined syndrome, such as cerebral palsy, Prune-Belly syndrome or posterior urethral valves; or retractile testes in controls. Based on an existing electronic health record (EHR) template used for over a decade at our institution, parents were routinely asked for information regarding birth history and their knowledge of first documentation of cryptorchid testicular position.

A surgeon co-investigator provided documentation of intraoperative testicular position, ipsilateral hernia sac, epididymal anomaly and/or existence of an ipsilateral testicular

appendage. The highest location of either testis was used in bilateral cases and defined as “proximal” for testes above the external inguinal ring (canalicular, abdominal) and “distal” for those below the external inguinal ring (superficial inguinal pouch, external ring, prescrotal, perineal). Participants with absent testes were excluded. Infant feeding type and breastfeeding duration data were obtained from a maternal and perinatal history questionnaire.

To best compare “congenital” and “acquired” forms of cryptorchidism, we adopted a conservative approach to limit misclassification of cases as acquired when cryptorchidism was present at birth but referral was delayed. We analyzed the cryptorchid age distribution and identified a major peak prior to age 2 followed by a lower but sustained frequency during childhood. This distribution was similar to that reported by Hack et al¹⁷ although our postnatal peak is earlier and the secondary peak less pronounced, possibly because many boys are referred to us for evaluation prior to 6 months of age. Based on our data and the recommendations of Bruijnen et al¹⁸ we categorized patients as having “congenital NSC” if surgery was performed at ≤24 months or if families or pediatricians of older boys reported undescended testes at birth but referral was delayed. Boys >24 months and without a prior cryptorchidism history were categorized as having “acquired NSC”.

Tissue samples

We collected samples of cremaster muscle and hernia sac (if present), tissues that are available from both cases and controls. Although the gubernaculum is considered the primary signaling target during testicular descent, postnatally it is a fibrous remnant that is dramatically different in composition from its prenatal form and is easily obtainable during orchidopexy but not hernia repair. A small portion of cremaster was removed during mobilization of the testis or inguinal hernia, and a portion of the excised hernia sac was collected. Tissues were preserved in RNA Later® (Ambion) until total RNA purification was performed using the RNeasy micro kit (Qiagen). cDNA generation was followed by Taqman-based real-time RT-PCR, and data analysis using the delta-delta threshold cycle method were performed. A single gubernacular tissue sample was run on all plates as a calibrator. Using cremaster and hernia sac samples we measured expression of *AR*, *ESR1* ($ER\alpha$), *ESR2* ($ER\beta$), *RFXP2* and *PR* (progesterone receptor) mRNA relative to vimentin as a loading control. In addition, we measured myosin heavy chain 1 (*MYH1*), which predominates in fast type IIx muscle fibers, myosin heavy chain 2 (*MYH2*), which predominates in fast type IIa muscle fibers, and slow-twitch/cardiac specific myosin heavy chain 7 (*MYH7*) in cremaster muscle samples only.

Statistical analysis

Continuous data were log-transformed and analyzed using Pearson’s correlation, ANOVA or 2-sample T tests. Results are presented as means of raw data $\pm$ SE. All analyses were performed using IBM SPSS v. 19.

Results

Clinical data

Clinical data for case and control subjects included in the study are shown in Table 1. As noted previously, boys with acquired cryptorchidism were significantly less likely to have testes proximal to the external inguinal ring ($p=0.019$) or an associated hernia or epididymal anomaly ($p<0.001$). In this selected subset of our case-control population, the frequency of soy formula use was significantly lower in the control group ($p=0.028$).

Receptor mRNA expression

Preliminary analysis revealed non-measurable levels of *RXFP2*, *ESR2* and *PR* mRNA in both cremaster muscle and hernia sac (Ct values > 35). *AR* and *ESR1* mRNA levels were measurable in both tissue types but levels were approximately 2–3 fold higher in cremaster than in hernia sac tissue from controls (Figure 1). *AR*, but not *ESR1* mRNA levels were significantly higher in control relative to NSC cremaster samples ($p < 0.01$), but were not significantly different in congenital vs. acquired subgroups. In contrast, both *AR* and *ESR1* levels were slightly but significantly lower in control hernia sac samples. *AR* and *ESR1* levels were correlated in both cremaster ($r^2 = .560$, $p < 0.001$) and hernia sac ($r^2 = .794$, $p < 0.001$). There were no significant differences in *AR* or *ESR1* mRNA levels in cryptorchid boys based on affected side (unilateral vs. bilateral), testis position (proximal vs. distal) or presence/absence of an associated inguinal hernia, epididymal abnormality or testicular appendage (data not shown).

Myosin heavy chain mRNA expression

In view of differences in *AR* expression between boys with hernia/hydrocele and boys with NSC, we analyzed muscle type-specific myosin heavy chain mRNAs in a subset of cremaster samples (Table 1). Initial analyses showed measurable expression of fast (*MYH2* but not *MYH1*) and slow/cardiac (*MYH7*) twitch mRNAs in all 3 groups (Figure 2). We noted a marked decrease in *MYH7* mRNA expression in congenital and acquired NSC cases relative to controls ($p = 0.002$). *MYH2* mRNA expression was higher in controls than in samples from cryptorchid boys, although the difference was significant only for the congenital group ($p < 0.05$). In individual samples, we observed a strong correlation between *MYH7* and *AR* levels ($r^2 = .751$, $p < 0.001$), weaker correlations between *MYH2* and *AR* ($r^2 = .255$, $p = 0.005$) and *MYH2* and age ($r^2 = -.189$, $p = 0.04$) and no correlation between levels of *MYH7* and *MYH2* ($r^2 = .150$, $p = 0.1$) or myosin heavy chain isoforms and *ESR1* ($r^2 < .1$, $p > .3$). Expression of *MYH7* was significantly higher and *ESR1* lower in boys with NSC who received soy formula in infancy ($p < 0.05$; Figure 3). We did not observe any other differences in mRNA expression in cremaster or in hernia sac relative to feeding history or cryptorchid phenotype (data not shown).

Discussion

In the present studies, we hypothesized that altered expression of hormone receptor and muscle type-specific mRNAs are present in target tissues of boys with NSC, potentially reflecting aberrant hormone-dependent signaling during development. Although we observed a reduction in *AR* expression in cremaster muscle of NSC cases as compared to boys with normal testicular descent, we observed increased expression of *AR* and *ESR1* in hernia sac of cases, suggesting that regulation of expression of these receptors is tissue-specific. While the factors that determine steroid receptor expression in these tissues remain unknown, cremasteric *Ar* mRNA expression is not regulated by androgens or INSL3 in neonatal rats.¹⁹ Consequently, altered *AR* mRNA expression does not necessarily reflect altered androgen exposure during fetal life and may not reflect protein levels but provided us the opportunity to screen relatively large numbers of minute tissue samples using a quantitative approach. Persistent postnatal differences in *AR* expression in these target tissues could reflect changes in the relative distribution of *AR*-expressing cell types, which may or may not depend on hormonal status. Notably, Kaftanovskaya et al have shown that *Ar* expression in mice with targeted deletion of *Rxfr2* (or downstream effectors β -catenin and *Notch1*) is reduced in migrating mesenchymal cells of the mouse gubernaculum, although it is unclear if these are fibroblasts or myoblasts.⁹ Therefore, *AR* expression in cremaster may in part reflect developmental activation of RXFP2 by INSL3. Moreover, as the patterns of differential mRNA expression we observed are similar for acquired and

congenital NSC, these subgroups of cryptorchidism may share a similar pathophysiology, as proposed by Rusnack et al in studies of testicular maldevelopment.²⁰

The target of AR signaling in the process of testicular descent has been controversial. Hutson and colleagues implicate genitofemoral sensory neurons or surrounding mammary tissue²¹ as possible indirect targets of androgen signaling during testicular descent in the rat. However, fetal rodent motoneurons do not express AR protein²² while AR is present in fetal rodent muscle and mesenchymal cells of the gubernaculum,^{8,9} albeit at lower levels than classic androgen-sensitive targets, and also in the human fetal gubernaculum.²³ Additional insight was recently provided by tissue-specific *Ar* knockout mouse models. Kaftanovskaya et al report that targeted ablation of *Ar* expression in gubernacular mesenchyme in mice leads to cryptorchidism that is associated with altered expression of muscle-specific genes.²⁴ In contrast, muscle specific knockout of *Ar* did not produce cryptorchidism, suggesting that androgens indirectly regulate cremaster muscle development.

Our data support previous observations that the cremaster muscle is predominantly a slow twitch muscle in humans but differ from the observations of Tanyel et al, who observed differences in fast twitch fiber diameter but not fiber type in cremaster muscle of cases (10 boys with cryptorchidism) and controls (10 boys with hernia).²⁵ However, their data suggest a slight increase in type 1 fibers in cryptorchid cases, a difference that may have failed to reach significance because of the small sample size. Similarly, Tanyel and colleagues reported an increase in *AR* mRNA and protein expression in cremaster muscles of cryptorchid boys as compared to boys with hernia (n=8 per group) using semiquantitative RT-PCR and immunostaining.²⁶ Differences in sample size, technique and/or patient populations may explain the discrepancies between their observations and the present data.

We identified a close correlation between *MYH7* and *AR* expression in cremaster muscle of both groups and a parallel reduction in both mRNAs in cases compared to controls. While *MYH7* and *MYH2* expression is reduced in boys with NSC as compared to controls, the reduction was disproportionately greater for the slow twitch isoform. The reduction in *MYH7* expression would not be predicted in the presence of reduced *AR* signaling, since both global and myocyte-specific inactivation of AR are associated with a switch from fast to slow fiber type predominance and androgens increase fast twitch myosin expression in striated muscle.¹⁶ However, as the cremaster shows marked sexual dimorphism, it may be unique in its hormonal sensitivity to fiber type determination. Alternatively, reduction of both *MYH7* and *MYH2* mRNA expression may reflect global impairment or delay in cremaster muscle development in NSC, factors that may play a role in failed testicular descent in humans as noted previously in a rat strain with inherited cryptorchidism.²⁷ Motor innervation is also known to regulate development of muscle, particularly slow twitch muscle²⁸ and may play a role in cremaster muscle development and in *AR* and myosin heavy chain expression.

Preliminary analysis of questionnaire data from our entire series of NSC cases and controls suggests an association between soy formula use and acquired NSC (Barthold et al, unpublished observations). The present data suggest differences in *MYH7* and *ESR1* mRNA expression in NSC based on exposure to soy formula, but the small number of individuals in the control precludes meaningful case-control comparisons. These data need to be validated in a larger series but are of potential interest, as infants receiving soy formula are exposed to high levels of several phytoestrogens with estrogen agonist activity.²⁹ Although these data do not establish an etiologic association between soy formula and cryptorchidism, they suggest that soy exposure may alter *MYH7* and/or *ESR1* expression in cremaster muscle.

Conclusions

We identified differences in *AR* and myosin isoform expression in boys with NSC as compared to a control group with hernia and/or hydrocele and completely descended testes, suggesting that cremaster muscle development is altered in association with failure of testicular descent. *ESR1* and *MYH7* were also differentially expressed relative to soy formula exposure in cryptorchid boys. These differences in gene expression may reflect aberrant hormonal signaling and/or innervation during development with secondary effects on function that lead to failed testicular descent. Further studies are needed to elucidate the etiology of NSC.

Acknowledgments

Support: 1R01HD060769-01A1 (NICHD), 1P20 RR20173-01 (NCRR) and Nemours Biomedical Research

References

1. Ashley RA, Barthold JS, Kolon TF. Cryptorchidism: pathogenesis, diagnosis, treatment and prognosis. *Urol Clin North Am*. 2010; 37:183. [PubMed: 20569797]
2. Ferlin A, Zuccarello D, Garolla A, et al. Hormonal and genetic control of testicular descent. *Reprod Biomed Online*. 2007; 15:659. [PubMed: 18062862]
3. Chacko JK, Barthold JS. Genetic and environmental contributors to cryptorchidism. *Pediatric Endocrinology Reviews*. 2009; 6:476. [PubMed: 19550382]
4. Emmen JM, McLuskey A, Adham IM, et al. Hormonal control of gubernaculum development during testis descent: gubernaculum outgrowth in vitro requires both insulin-like factor and androgen. *Endocrinology*. 2000; 141:4720. [PubMed: 11108287]
5. Kubota Y, Temelcos C, Bathgate RA, et al. The role of insulin 3, testosterone, Mullerian inhibiting substance and relaxin in rat gubernacular growth. *Mol Hum Reprod*. 2002; 8:900. [PubMed: 12356938]
6. Scott DJ, Fu P, Shen PJ, et al. Characterization of the rat INSL3 receptor. *Annals of the New York Academy of Sciences*. 2005; 1041:13. [PubMed: 15956681]
7. McKinnell C, Sharpe RM, Mahood K, et al. Expression of insulin-like factor 3 protein in the rat testis during fetal and postnatal development and in relation to cryptorchidism induced by in utero exposure to di (n-Butyl) phthalate. *Endocrinology*. 2005; 146:4536. [PubMed: 16037377]
8. Staub C, Rauch M, Ferriere F, et al. Expression of estrogen receptor ESR1 and its 46-kDa variant in the gubernaculum testis. *Biology of Reproduction*. 2005; 73:703. [PubMed: 15944240]
9. Kaftanovskaya EM, Feng S, Huang Z, et al. Suppression of insulin-like3 receptor reveals the role of beta-catenin and Notch signaling in gubernaculum development. *Molecular Endocrinology*. 2011; 25:170. [PubMed: 21147849]
10. Welsh M, Saunders PT, Fiskin M, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. *Journal of Clinical Investigation*. 2008; 118:1479. [PubMed: 18340380]
11. Husmann DA, McPhaul MJ. Time-specific androgen blockade with flutamide inhibits testicular descent in the rat. *Endocrinology*. 1991; 129:1409. [PubMed: 1678699]
12. Spencer JR, Torrado T, Sanchez RS, et al. Effects of flutamide and finasteride on rat testicular descent. *Endocrinology*. 1991; 129:741. [PubMed: 1677329]
13. Brokken LJ, Adamsson A, Paranko J, et al. Antiandrogen exposure in utero disrupts expression of desert hedgehog and insulin-like factor 3 in the developing fetal rat testis. *Endocrinology*. 2009; 150:445. [PubMed: 18772241]
14. Bay K, Main KM, Toppari J, et al. Testicular descent: INSL3, testosterone, genes and the intrauterine milieu. *Nat Rev Urol*. 2011; 8:187. [PubMed: 21403659]
15. Barthold JS. Undescended testis: current theories of etiology. *Current Opinion in Urology*. 2008; 18:395. [PubMed: 18520761]

16. de Gendt K, Verhoeven G. Tissue- and cell-specific functions of the androgen receptor revealed through conditional knockout models in mice. *Mol Cell Endocrinol*. 2011
17. Hack WW, Meijer RW, Van Der Voort-Doedens LM, et al. Previous testicular position in boys referred for an undescended testis: further explanation of the late orchidopexy enigma? *BJU Int*. 2003; 92:293. [PubMed: 12887487]
18. Bruijnen CJ, Vogels HD, Beasley SW. Review of the extent to which orchidopexy is performed at the optimal age: implications for health services. *ANZ Journal of Surgery*. 2008; 78:1006. [PubMed: 18959702]
19. Feng S, Bogatcheva NV, Truong A, et al. Over expression of insulin-like 3 does not prevent cryptorchidism in GNRHR or HOXA10 deficient mice. *Journal of Urology*. 2006; 176:399. [PubMed: 16753450]
20. Rusnack SL, Wu HY, Huff DS, et al. The ascending testis and the testis undescended since birth share the same histopathology. *Journal of Urology*. 2002; 168:2590. [PubMed: 12441991]
21. Lie G, Hutson JM. The role of cremaster muscle in testicular descent in humans and animal models. *Pediatr Surg Int*. 2011; 27:1255. [PubMed: 22038274]
22. Forger NG. The organizational hypothesis and final common pathways: Sexual differentiation of the spinal cord and peripheral nervous system. *Hormones & Behavior*. 2009; 55:605. [PubMed: 19446077]
23. Kohler B, Delezoide AL, Boizet-Bonhoure B, et al. Coexpression of Wilms' tumor suppressor 1 (WT1) and androgen receptor (AR) in the genital tract of human male embryos and regulation of AR promoter activity by WT1. *Journal of Molecular Endocrinology*. 2007; 38:547. [PubMed: 17496156]
24. Kaftanovskaya EMHZ, Barbara AM, et al. Cryptorchidism in mice with an androgen receptor ablation in gubernaculum testis. *Mol Endocrinol*. 2012; 26 epub Apr 2012.
25. Tanyel FC, Erdem S, Altunay H, et al. Distribution and morphometry of fiber types in cremaster muscles of boys with inguinal hernia or undescended testis. *Pathol Res Pract*. 2000; 196:613. [PubMed: 10997735]
26. Tanyel FC, Yuzbasioglu A, Kocaefe C, et al. Androgen receptor immunostaining and androgen receptor messenger ribonucleic acid expression are increased in cremaster muscles associated with undescended testis. *Urology*. 2006; 67:855. [PubMed: 16566985]
27. Barthold JS, McCahan SM, Singh AV, et al. Altered expression of muscle- and cytoskeleton-related genes in a rat strain with inherited cryptorchidism. *Journal of Andrology*. 2008; 29:352. [PubMed: 18222913]
28. Schiaffino S. Fibre types in skeletal muscle: a personal account. *Acta Physiologica*. 2010; 199:451. [PubMed: 20353491]
29. McCarver G, Bhatia J, Chambers C, et al. NTP-CERHR expert panel report on the developmental toxicity of soy infant formula. *Birth Defects Res B Dev Reprod Toxicol*. 2011; 92:421. [PubMed: 21948615]

Figure 1.

Expression of *AR* and *ESR1* mRNA in cremaster muscle (A) and hernia sac (B) of boys with congenital (n=62) or acquired (n=58) NSC or hernia/hydrocele controls (n=58); *p < 0.05, **p < 0.01 relative to control group.

Figure 2. Expression of *MYH7* and *MYH2* in cremaster muscle of boys with congenital or acquired NSC and hernia/hydrocele controls; * $p < 0.05$ and *** $p = 0.002$.

Figure 3.
Levels of *MYH7* mRNA are increased and *ESR1* mRNA reduced in cremaster samples from boys with NSC who received soy formula feeding in infancy; * $p < 0.05$.

Patient and sample data from boys with hernia/hydrocele (controls), and congenital and acquired nonsyndromic cryptorchidism (NSC) cases.

Table 1

	Control	NSC		p value
		Congenital	Acquired	
Patients	84	79	66	
Samples				
Cremaster	58	62	58	
Hernia sac	51	50	23	
Age (yrs)	3.83 ± 2.67	4.76 ± 3.60		NS
Birth weight (gm)	3553 ± 510	3448 ± 601	3528 ± 541	NS
Surgical data				
Bilateral		19 (25%)	19 (28%)	NS
Proximal to external ring		31 (40%)	14 (21%)	0.019
Hernia sac		67 (87%)	35 (52%)	<0.001
Epididymal anomaly		48 (63%)	19 (29%)	<0.001
Appendage		60 (78%)	44 (67%)	NS
Feeding data				
Soy formula	5 (7%)	12 (17%)	14 (23%)	0.028
No soy formula	68 (93%)	60 (83%)	46 (77%)	