Ageing of the Female Pelvic Floor

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Mario Joao Gomes, 1960-2015
General Secretary of MIPS

Strong Pelvic Floor

“ We define the GERIPAUSE as that point LATER in the postmenopausal years in which estrogen administration does NOT give the anticipated results due to chronic hypoestrogenicity”

Turner RJ, Kerber IJ. Eu-estrogenemia, WHI, timing and the “geripause”. Int Urogynecol J 2008; 19: 1461-3.

The Geripause

Study of the “geripause” is receiving much attention lately for 3 main reasons:

1. Global increase in female life expectancy because of
   a- Improved health services
   b- Continuing reduction of adult mortality
   c- Progressive transition from high to low fertility
   d- Recent socioeconomic affluence in most countries.

   This unprecedented demographic change allowed women to experience menopausal manifestations during one third of their life span and reach the geripause.

World Population Ageing 2013. United Nations Department of Economic and Social Affairs, Population Division. United Nations, 2013.

The Geripause

2. Process of “medicalization” and dominance of health as a cultural preoccupation in recent societies (More women are motivated by personal concerns and cultural forces to take control of the effects of menopause and/or ageing on their bodies- use of HRT).

3. Growing public and medical concern about the serious adverse effects of HRT in geripausal women (WHI Trial).

Rizk DEE. Fahim MA. Ageing of the female pelvic floor: Towards treatment “a la carte” of the “geripause” . Int Urogynecol J 2008; 19: 455-8.

Rossouw JE, et al, Writing Group for the Women's Health Initiative Investigators: Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002; 288: 321-33.

Etiology

- As yet, there is NO consensus whether deterioration of pelvic floor support after menopause is caused by:
  a- Normative ageing
  b- Falling circulating estrogen levels (OVARIAN FAILURE)
  c- Combination of both factors.

- To date, a significant biomedical knowledge gap exists about the relationships between menopause and ageing that is stereotyped by MEDIA PORTRAYAL and LAY PERCEPTIONS of both processes as SYNONYMOUS.

Wyman JF, et al. Shaping future directions for incontinence research in aging adults: executive summary. Nurs Res 2004; 53 (Suppl 1): S1-10.

Tariq MH. Geriatric fecal incontinence. Clin Geriatr Med 2004; 20: 571-87.

Hypoestrogenism?

- It is widely believed that estrogen deprivation at the climacteric is primarily responsible for support-related pelvic floor dysfunction (POP,SUI, FI) in geripausal women.

- This assumption was based on the detection of estrogen RECEPTORS in components of continence-maintaining and supportive pelvic floor structures in experimental animals and pre-menopusal women.

Tannenbaum C, et al. The aging pelvic floor. In: Crocos J, Schick E, editors, The urinary sphincter. New York: Marcel Dekker Inc.; 2001:175-81.

Plas E, Daha LK. Hormone replacement therapy for the aging bladder. In: Plas E et al, editors. The aging bladder. Wien, New York: Springer-Verlag; 2004. p. 137-54.

Estrogen Replacement Therapy?

- ERT had been extensively used to prevent or restore the decline in pelvic floor support and/or deterioration of urinary and fecal control after menopause.

- However, this was accepted ***ad verbatim*** with NO critical analysis of the long-term cure rates or evidence-based improvement in clinical outcome following treatment.

Donnelly V, et al. The influence of oestrogen replacement on faecal incontinence in postmenopausal women. Br J Obstet Gynaecol 1997; 104: 311-5.

Hextall A. Oestrogens and lower urinary tract function. Maturitas 2000; 36: 83-92.

Fornell EU, et al. Factors associated with pelvic floor dysfunction with emphasis on urinary and fecal incontinence and genital prolapse: an epidemiologic study. Acta Obstet Gynecol Scand 2004; 83: 383-9.

Evidence-Based Medicine

- Systematic review of epidemiological studies consistently found that AGE is the strongest risk factor while the role of menopause remains uncertain.

- A recent meta-analysis and a large prospective RCT showed that ERT paradoxically INCREASED the risk and severity of UI in continent and symptomatic postmenopausal women.

van Geelen J M, Hunskaar S. The epidemiology of female urinary incontinence. Eur Clinics Obstet Gynaecol 2005; 1: 3-11.

Pretlove SJ, et al. Prevalence of anal incontinence according to age and gender: a systematic review and meta-regression analysis. Int Urogynecol J 2006; 17: 407-17.

Steinauer JE, et al for the Heart and Estrogen/Progestin Replacement Study Research Group. Post menopausal hormone therapy: does it cause incontinence? Obstet Gynecol 2005; 106: 940-5.

Hendrix SL, et al. Effects of estrogen with and without progestin on urinary incontinence. JAMA 2005; 293: 935-48.

Biological Ageing?

- Experimental, epidemiological and clinical data suggest that age-related changes normally occurring during menopausal transition may **contribute** to the decline in pelvic floor support.

- These “intrinsic” age-related changes are INDEPENDENT of the altered ovarian hormonal milieu of the menopause.

Moalli, P , et al. Hormones restore biomechanical properties of the vagina and supportive tissues after surgical menopause in young rats. Am J Obstet Gynecol 2008; 199:161.e1-8.

Pfisterer MH, et al . The effect of age on lower urinary tract function: a study in women. J Am Geriatr Soc 2006; 54: 405-12.

Ng SC, Chen GD. Age effects on anorectal pressure in anal continent women with lower urinary tract dysfunction. Int Urogynecol J 2007; 18: 295-300.

Pierce LM, et al. Levator ani muscle and connective tissue changes associated with pelvic organ prolapse, parity, and aging in the squirrel monkey: a histologic study. Am J Obstet Gynecol 2007; 197: 60.e1-9.

Female Geriatric Disorders

- A *bona fide* effect of biological ageing on the postmenopausal pelvic floor CANNOT be excluded.

- Changes induced by ageing are widely accepted in the pathogenesis of other degenerative female geriatric disorders such as bone, joint, musculoskeletal, cardiovascular and neurological diseases !!

Waetjen LE, et al for the Study of Women's Health Across the Nation (SWAN). Factors associated with worsening and improving urinary incontinence across the menopausal transition. Obstet Gynecol 2008; 111: 667-77.

Hall JE. Neuroendocrine physiology of the early and late menopause. Endocrinol Metab Clin North Am 2004; 33: 637-59.

Gender Differences

- Ageing is an evolutionary event of progressive processes affecting molecules, cells and the whole organism caused by free radicals, non-enzymatic glycosylation and apoptosis.

- Ageing is controlled by endocrine hormones particularly GH, estrogens and androgens with a SIGNIFICANT gender differences in the pace of development of ageing.

- Some neural functions gradually decline with ageing but are accelerated in women after menopause with a significantly greater deterioration in females than males.

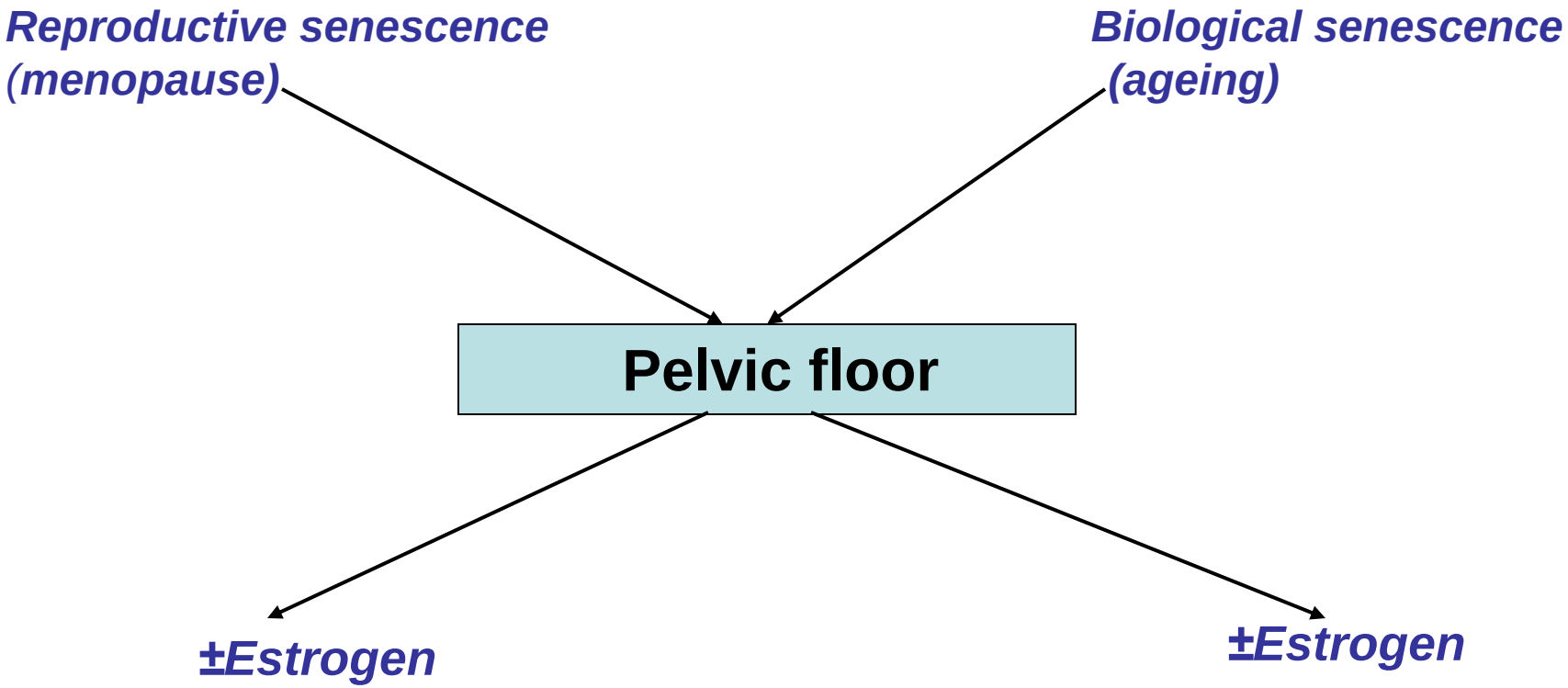
Siek G. Physiology of aging. J Appl Physiol 2003; 95: 1333-4.

Markou A, et al. Estrogens and brain function. Hormones 2005; 4: 9-17.

Lelbach A, et al. The molecular biology of ageing- therapeutic interventions? Orv Hetil 2006; 147: 41-8.

Natural model par excellence

Geripausal women



Animal Models

- The human pelvic floor differs structurally and functionally from that of quadrupeds because the levator muscle primarily functions to support the tail without a major role in supporting pelvic organs.

- However, the anatomy of the urethra, anal canal and pelvic floor muscles in **the female rat** is sufficiently similar *in toto* to that of the human female to serve as a model for morphological studies.

- Laboratory animals which are OLD and those with surgical-induced ovarian failure (ovariectomy- OVX) are useful PROXY models to study this phenomenon.

Fisher 344 Rats

- Fisher 344 rats are particularly suitable for ageing research because the mean survival time of this colony is prolonged (30 months) compared to other rat models.

- Puberty occurs between 2-4 months of age in females while ageing changes start around 15-16 months of age allowing the study of the effects of OVX and ageing in various pelvic floor tissues over a wider age range.

Rizk DEE, et al. The effect of ovariectomy on biomarkers of urogenital ageing in old versus young adult rats. Int Urogynecol J 2007; 18: 1077-85.

Moalli PA, et al. A rat model to study the structural properties of the vagina and its supportive tissues. Am J Obstet Gynecol 2005; 192: 80-8.

Surrogate Biomarkers of Pelvic Floor Ageing

1- Number of vascular plexuses
2- Collagen type I/III ratio in the submucosa of the urethra and anal canal.
3- Expression of cytoplasmic $p27^{kip1}$ protein
4- Proportion of isomyosin or myosin heavy chain (MHC) type I/type II
5- Ultrastructural changes in satellite cell number in the striated pelvic floor muscles.

Kerkhof MH, et al: Changes in connective tissue in patients with pelvic organ prolapse-a review of the current literature. Int Urogynecol J 2009; 20: 461-74.

Siek GC. Physiology of aging. J Appl Physiol 2003; 95:1333-4.

Rizk DEE, et al. The effect of ovariectomy on biomarkers of urogenital ageing in old versus young adult rats. Int Urogynecol J 2007; 18: 1077-85.

Number of submucosal urethral and anal canal vessels

- The number of peri-urethral and peri-anal submucosal vessel ‘cushion’ is an important component of intrinsic urethral and anal canal function in women responsible for maintaining continence.

- The number of vessels can be counted objectively by light microscopy.

Gordon PH. Anorectal anatomy and physiology. Gastroentrol Clin North Am 2001; 30: 1-9.

Verelst M, et al. Computerised morphometric study of the paraurethral tissue in young and elderly women. Neurourol Urodynam 2002; 21: 529-33.

Number of submucosal urethral and anal canal vessels

- A well-developed estrogen-dependent submucosal venous plexus has been described in the female urethra and anal canal of rats.

- The urethral and anal vascular counts in old Wistar female rats were significantly reduced compared to young adult animals and after OVX in both groups. Estrogen restored vessel counts *only* in *young* OVX animals.

Rizk DEE, et al: Effects of ovariectomy and hormone replacement on collagen and blood vessels of the urethral submucosa of rats. Urol Res 2003; 31: 147- 51.

Mensah-Brown EP, Rizk DEE, et al: Effects of ovariectomy and hormone replacement on submucosal collagen and blood vessels of the anal canal of rats. Colorectal Dis 2004; 6: 481-7.

Rizk DEE, et al. Estrogen and ghrelin increase the number of submucosal urethral and anal canal blood vessels in ovariectomized rats. Urology 2005; 66: 1343-8.

Collagen type I/III ratio in the submucosa of the urethra and anal canal

- Collagen fibers type I and III are located in the urethral and anal canal submucosal connective tissue where they significantly influence biological continence function.

- Qualitative changes can be measured by immuno-histochemistry and quantitative changes by Western blot analysis.

Rizk DEE, et al. Effects of ovariectomy and hormone replacement on collagen and blood vessels of the urethral submucosa of rats. Urol Res 2003; 31: 147-151.

Mensah-Brown EP, et al: Effects of ovariectomy and hormone replacement on submucosal collagen and blood vessels of the anal canal of rats. Colorectal Dis 2004; 6: 481-7.

Goepel C, et al. Periurethral connective tissue status of postmenopausal women with genital prolapse with and without stress incontinence. Acta Obstet Gynecol Scand 2003; 82: 659-64..

Collagen type I/III ratio in the urethral and anal canal submucosa

- Changes in type I/III collagen ratio correlates with changes in the mechanical properties of the urethra and anal canal (type I is more rigid and supportive- type III contributes more to elastic properties).

- **Decreased type I/III collagen ratio** results in tissue laxity and is clinically associated with loss of compliance or decreased resting pressure.

- Decreased ratio occurs with ageing in female monkeys and after OVX in rats.

Kerkhof MH, et al: Changes in connective tissue in patients with pelvic organ prolapse—a review of the current literature. Int Urogynecol J 2009; 20: 461-74.

Mensah-Brown EP, et al: Effects of ovariectomy and hormone replacement on submucosal collagen and blood vessels of the anal canal of rats. Colorectal Dis 2004; 6: 481-7.

p27 ^{kip1} in the striated muscles

- p27^{kip1} (cycline-dependent KI required for cell cycle arrest) regulates striated muscle cell differentiation and apoptosis and is one of the specific cellular markers of ageing.

- Cytoplasmic expression of this marker in striated muscles can be measured using Western blot analysis.

- Elderly postmenopausal patients with SUI or FI show strong expression of p27^{kip1} in the levator muscle cells associated with microscopic muscle shrinking compared to younger postmenopausal or pre-menopusal patients.

Welle S, et al. Skeletal muscle gene expression profiles in 20-29 year old and 65-71 year old women. Exp Gerontol 2004; 39: 368-77.

Bukovsky A, et al. Abnormal expression of p27kip1 protein in levator muscle of aging women with pelvic floor disorders- a relationship to the cellular differentiation and degeneration. BMC Clin Pathol 2004; 1:4

MHC type I/II in the striated muscles

- The proportion of type I (slow) to type II (fast) MHC muscle fibers determines muscle strength and contractile characteristics.

- This proportion can be measured by immuno-histochemistry, histochemistry (ATPase staining) or Western blot analysis.

Zhu W, et al. Morphologic study on levator ani muscle in patients with pelvic organ prolapse and stress urinary incontinence. Int Urogynecol J 2005; 16: 401-4.

Yiou R, et al. The pathophysiology of pelvic floor disorders: evidence from a histometric study of the perineum and a mouse model of rectal prolapse. J Anat 2001; 199: 599-607.

MHC type I/II in the striated muscles

- **A higher proportion of MHC I/II** is associated with SUI.
- OVX increases MHC I/II in pelvic floor muscles of rats and this is reversed by estrogen.
- In aged Fisher 344 female rats, the ratio is also increased (isoform transition from II to I).

Piccone J, et al. Effect of oestrogen on myofibre size and myosin expression in growing rats. Exp Physiol 2005; 90: 87-93.

Russell B, et al. Morphometry of the aging female rat urethra. Int Urogynecol J 1996; 7: 30-6.

Satellite cell number/muscle cell

- Satellite cells are quiescent precursors of the myofibers, their number represents capacity for repair and regeneration of striated muscles including pelvic floor muscles.
- Ageing induces massive depletion of this number.
- The satellite cell number/muscle cell can be measured by electron microscopy.
- Significant depletion of satellite cells was observed in the levator muscle of senile rats compared to juvenile rats.

Heeple R. Dividing to keep muscle together: the role of satellite cells in aging skeletal muscle. Sci Aging Knowledge Environ 2006; 18: pe3.

Nnodium JO. Satellite cell numbers in senile rat levator ani muscle. Mech Ageing Dev 2000; 112: 99-111.

Anti-Ageing Drugs

- GH has an anti-ageing function in humans because the circulating levels show age-related changes (increase at puberty and gradual decline with ageing) and is widely used in ageing research.

- The most potent GH secretagogue is ghrelin, a recently discovered 28 amino-acid peptide hormone isolated from the rat and human stomach.

- Ghrelin also has reproductive functions in women since its secretion/function appears to be regulated by estrogen.

Kojima M, Kangawa K. Drug Insight: the functions of ghrelin and its potential as a multitherapeutic hormone. Nature Clin Pract Endocrinol Metab 2006; 2: 80-8.

Chu MC, et al. Insulin resistance in postmenopausal women with metabolic syndrome and the measurements of adiponectin, leptin, resistin, and ghrelin. Am J Obstet Gynecol 2006; 194: 100-4.

Hypothesis

- *ESTROGEN-INDEPENDENT biological ageing changes occur in the urethra, anal canal and levator ani of an animal model of OLD OVX rats and that these changes can be reversed by anti-ageing therapy such as ghrelin.*

- *This work will provide experimental evidence for non-estrogen mediated effects on the pelvic floor apparatus in geripausal women with a potential for improved and safer pharmacological intervention.*

Rizk DEE. Fahim MA. Ageing of the female pelvic floor: Towards treatment "a la carte" of the "geripause". Int Urogynecol J 2008; 19: 455-8.

Experiments

- 48 Fisher 344 *(Harlan Industries, IN, USA)* female rats (18- and 3-months-old, n=24×2) underwent bilateral OVX. 12 more age-matched animals underwent sham surgery (n=6×2).

- After 30 days, study rats were assigned to 4 subgroups (n=6):
  1- Group 1: 17-ß estradiol 10 µg/kg/day,
  2- Group 2: ghrelin 2µg/kg/day
  3- Group 3: both drugs
  4- Group 4: a vehicle.

- All drugs were administered IP daily for 42 days.

Methods

- External urethral and anal sphincters and levator muscle specimens were frozen in liquid nitrogen for measurement of cytoplasmic $p27^{kip1}$ and MHC I protein expression by Western blot analysis.

- Frozen tissue from the urethra and anal canal were used for Western blot analysis of submucosal collagen types I and III.

- Signal intensity and image analysis were evaluated using Image Analyser (BioDoc analyser 20).

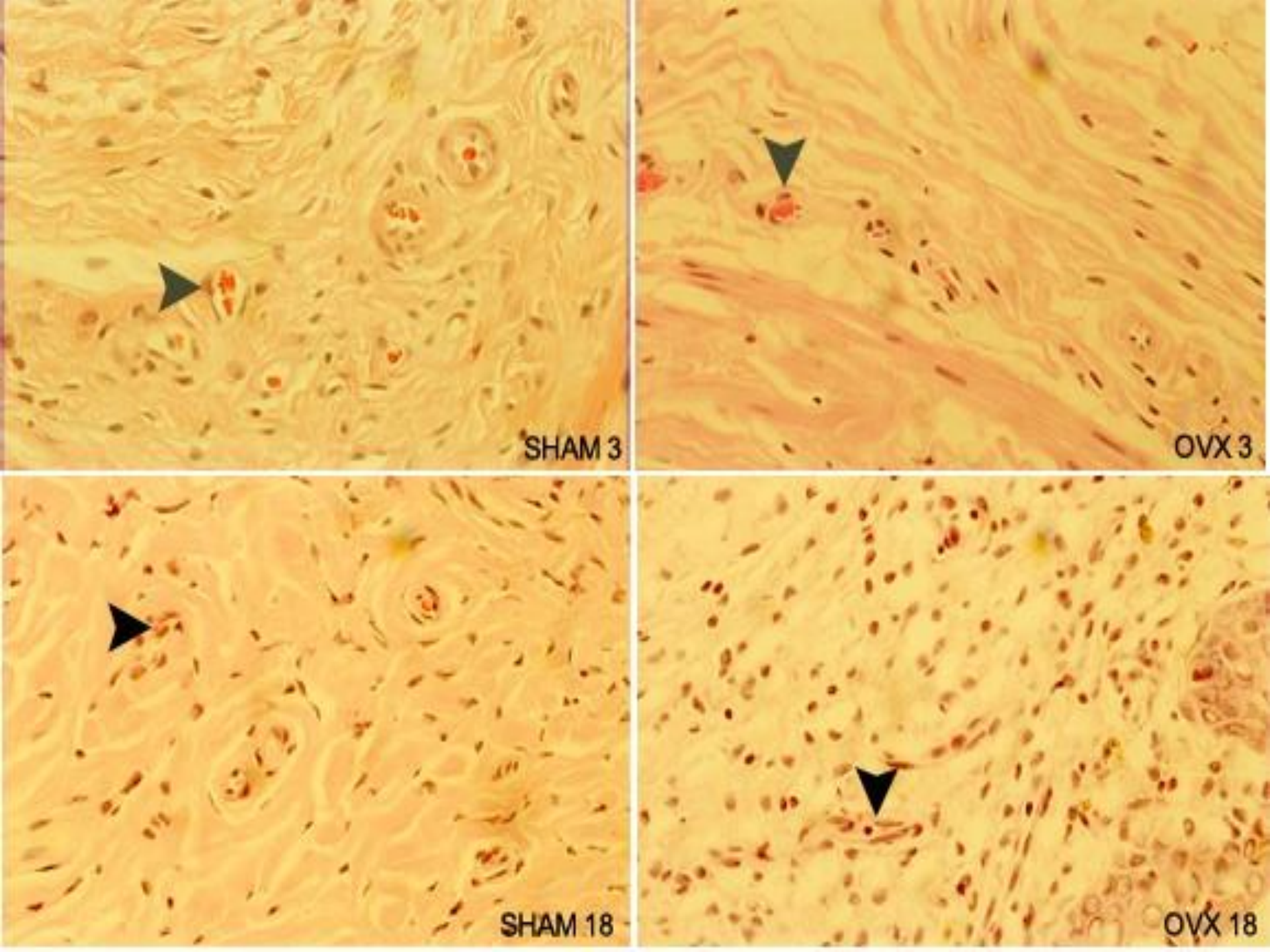
Methods

- Second urethral, anal canal and levator muscle specimens were fixed and stained with H& E.
- Submucosal blood vessels were counted by light microscopy in 5 randomly-selected fields x400.
- Immuno-histochemistry of ghrelin receptors was performed in fixed urethral, anal canal and striated pelvic floor muscle specimens.
- Radioimmunoassay of circulating GH was performed.

Results- I

- Mean submucosal urethral and anal vascular counts were significantly reduced in SHAM 18 compared to SHAM 3 rats.

- Mean submucosal urethral and anal vascular counts were significantly reduced after OVX only in the OVX 18 group but not in the OVX3 group.


SHAM 3
OVX 3
SHAM 18
OVX 18

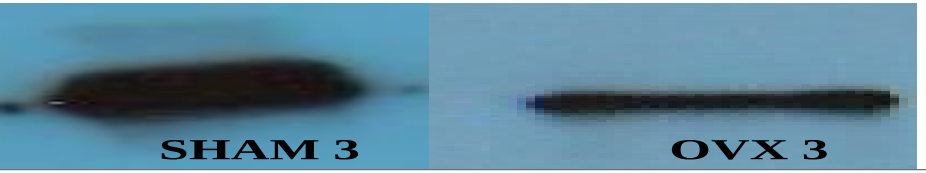
Results- II

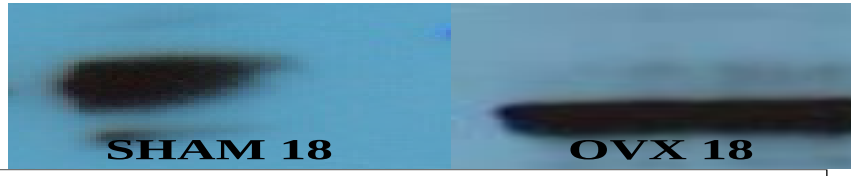
- **Urethral** mean type I/III collagen signal intensity ratio was significantly reduced after OVX in OVX 3 and OVX 18 groups. The difference between SHAM 3 and SHAM 18 groups was not significant.

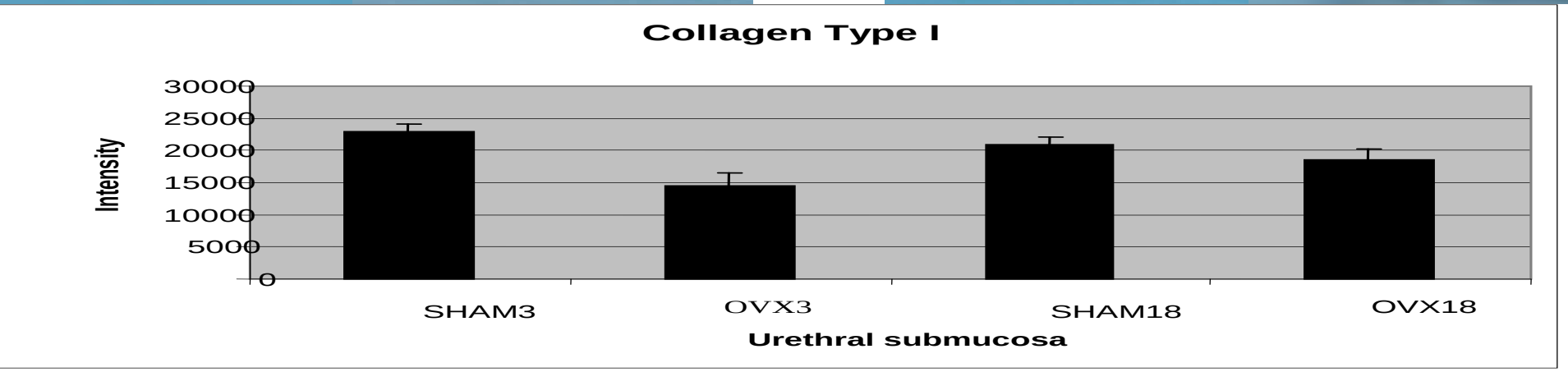
- **Anal canal** mean type I/III ratio was significantly reduced in SHAM 18 compared to SHAM 3 group. The decrease in ratio after OVX was not significant.

- Therefore, type I/III collagen signal intensity ratio significantly decreased after OVX or ageing in the urethra and anal canal, respectively.

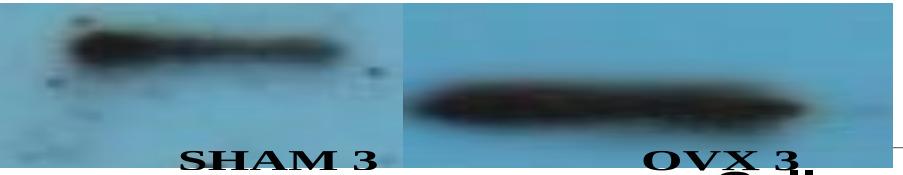
a- Collagen type I

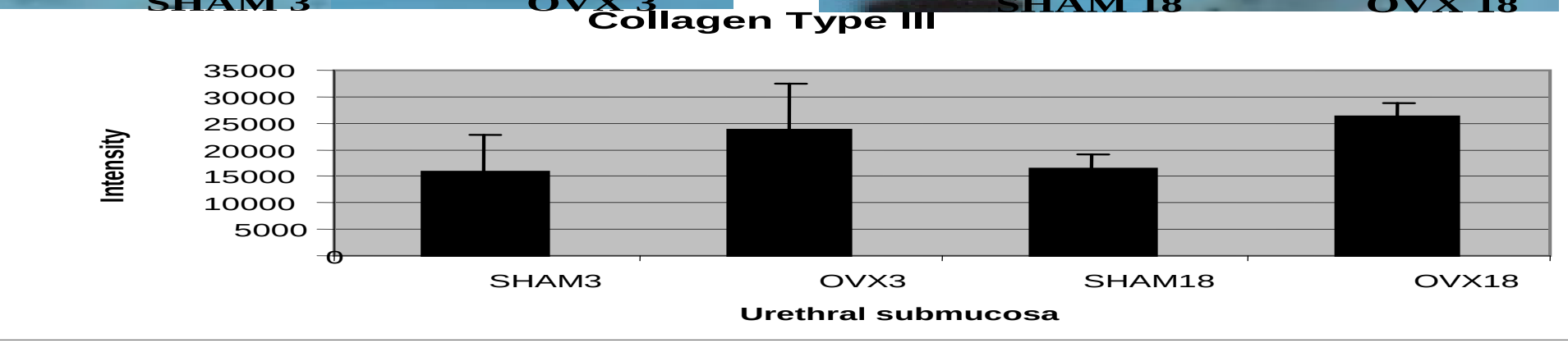






b- Collagen type III



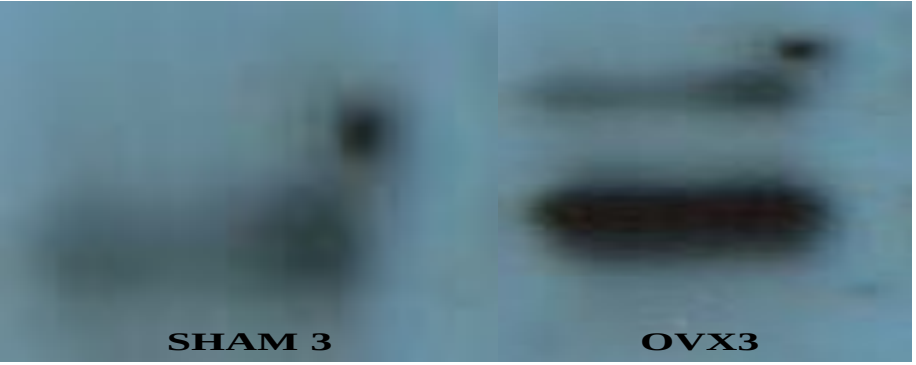


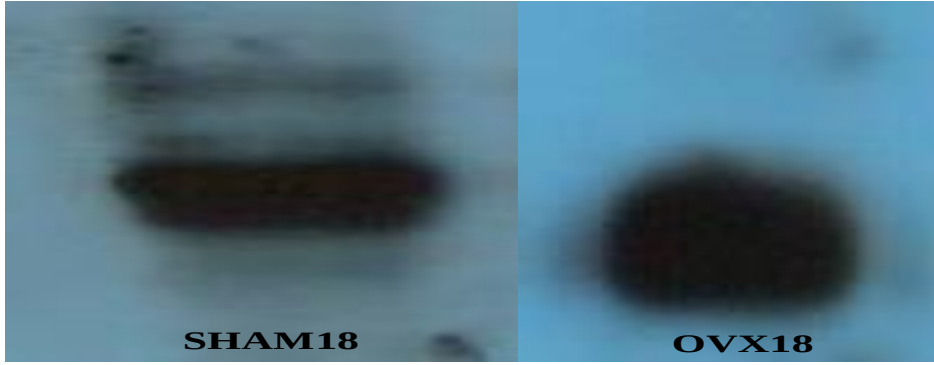
Results- III

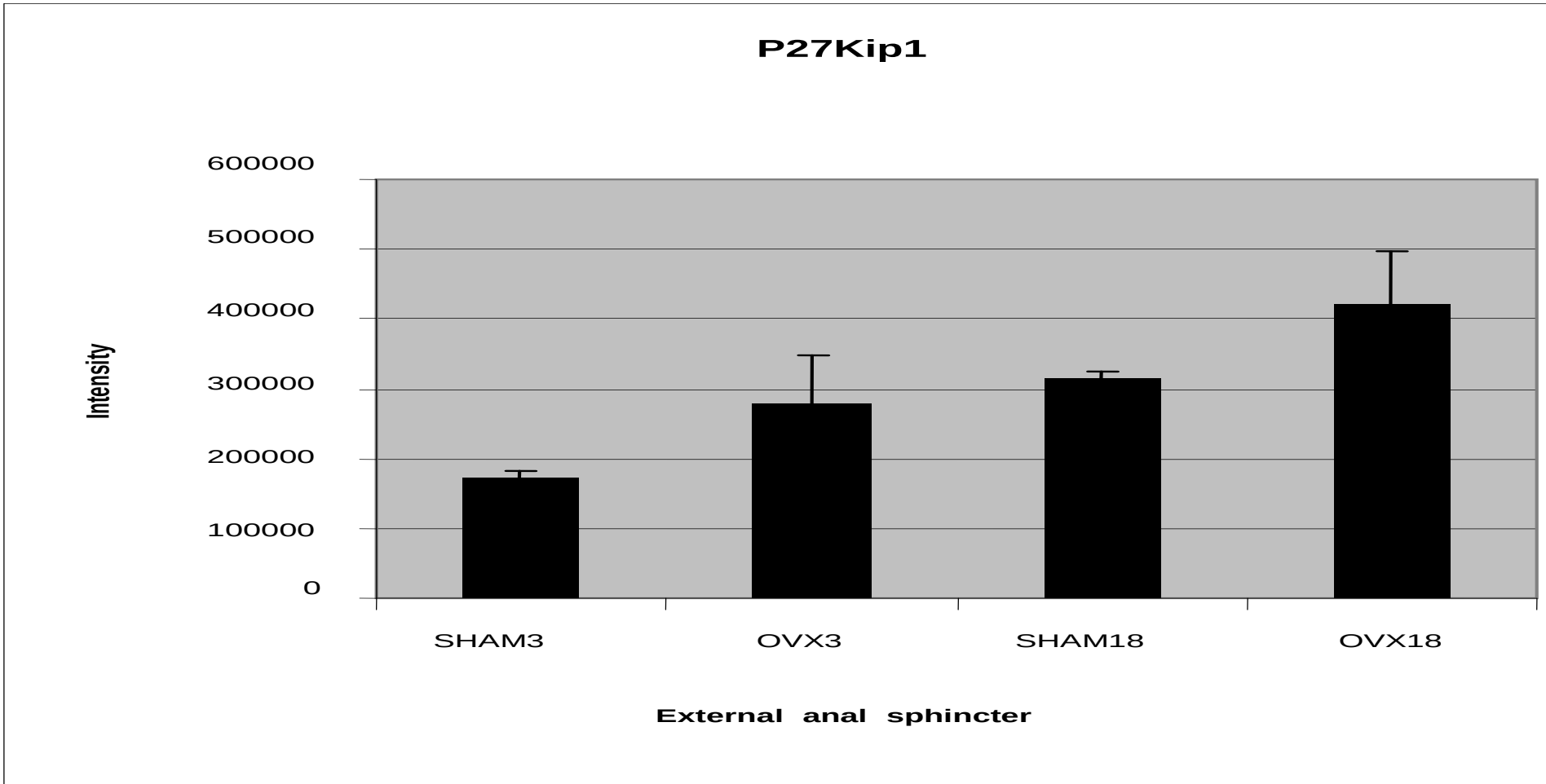
- Signal intensity of cytoplasmic $p27^{kip1}$ was significantly increased in SHAM 18 compared to SHAM 3 rats in the urethral sphincter, anal sphincter and levator.

- A significant increase in intensity in all muscles was observed after OVX in OVX 18 rats.

- OVX increased $p27^{kip1}$ intensity in the OVX 3 group but the difference was significant only in the urethral and anal sphincters.


SHAM 3
OVX3


SHAM18
OVX18

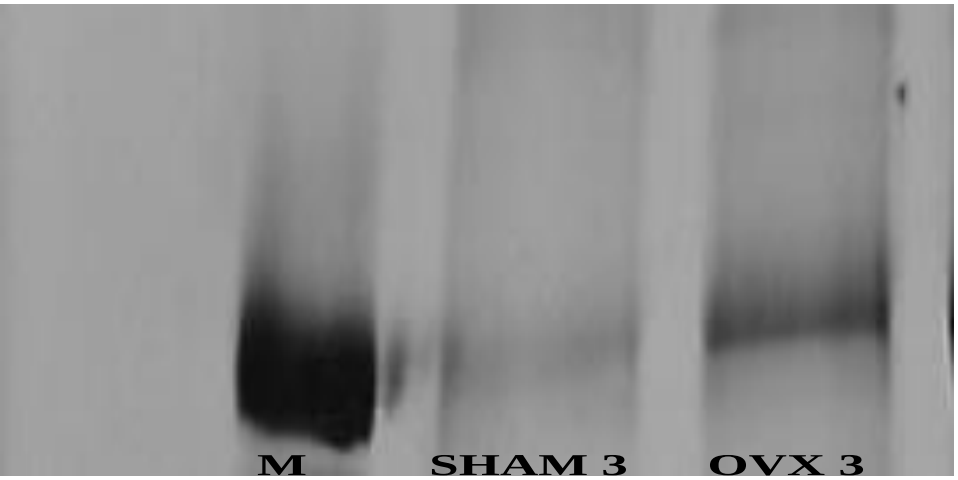

P27Kip1
600000
500000
400000
300000
200000
100000
0
Intensity
SHAM3
OVX3
SHAM18
OVX18
External anal sphincter

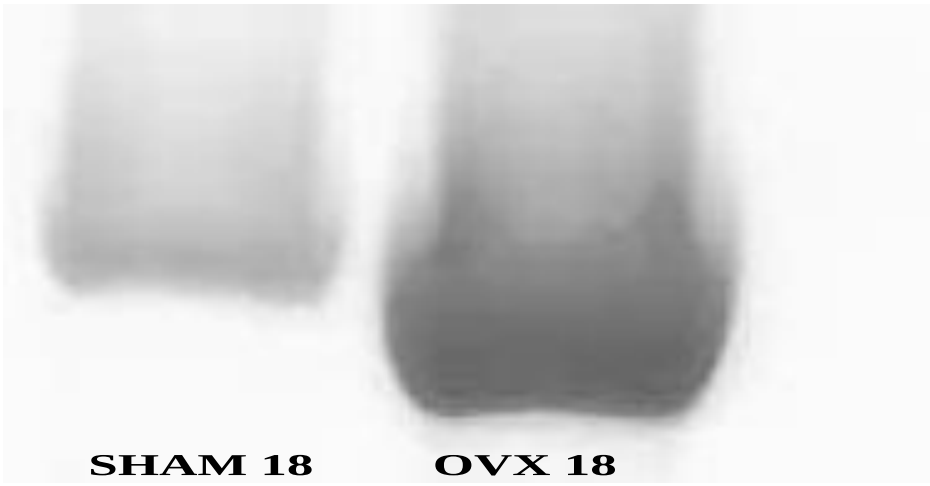
Results- IV

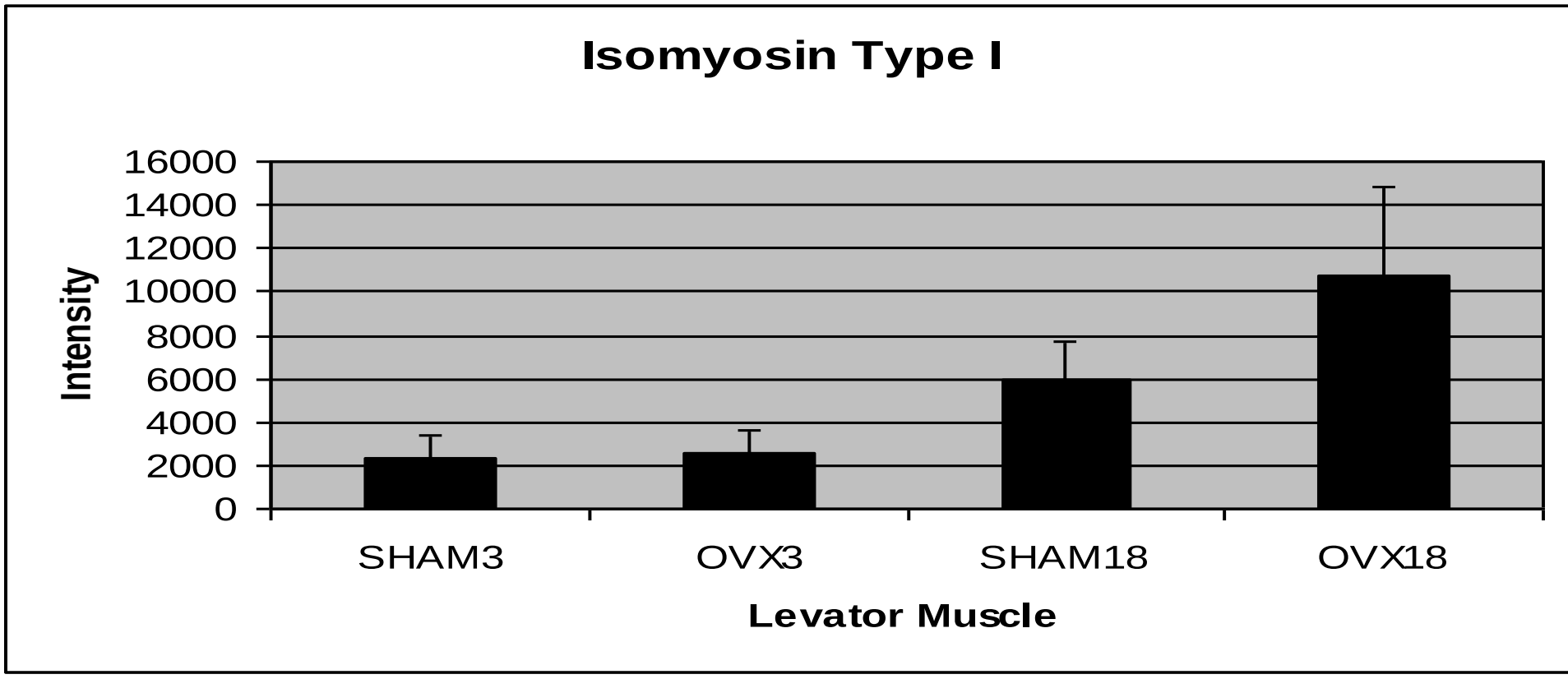
- Isomyosin (MHC) type I signal intensity was significantly increased in SHAM 18 compared to SHAM 3 rats in the urethral sphincter, anal sphincter and levator.

- A significant increase in intensity in all muscles was observed after OVX in OVX 18 rats particularly in the levator muscle.

- OVX increased signal intensity in the OVX 3 group but the difference was not significant in any muscle.


M
SHAM 3
OVX 3

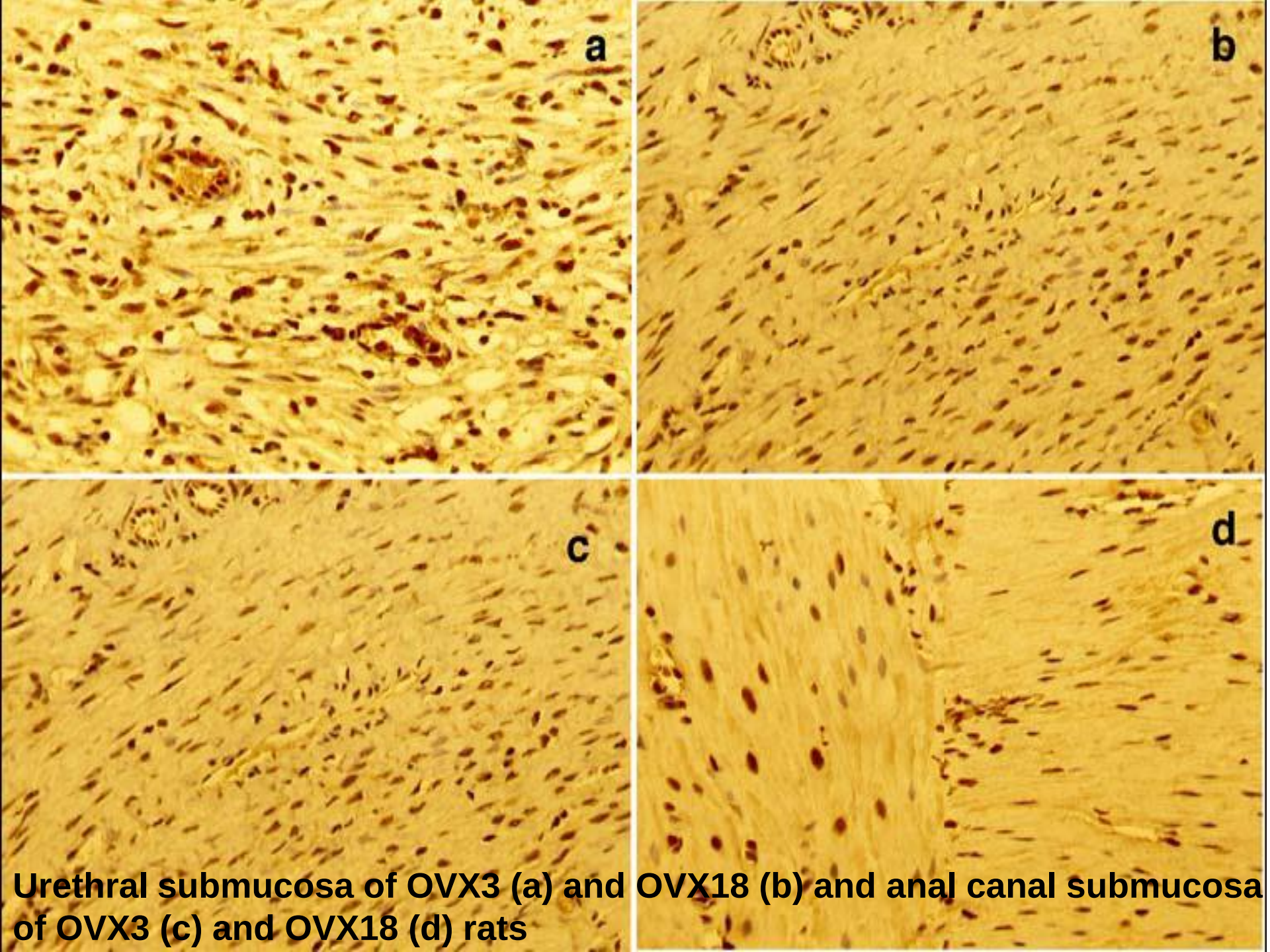

SHAM 18
OVX 18


Isomyosin Type I
Intensity
16000
14000
12000
10000
8000
6000
4000
2000
0
SHAM3
OVX3
SHAM18
OVX18
Levator Muscle

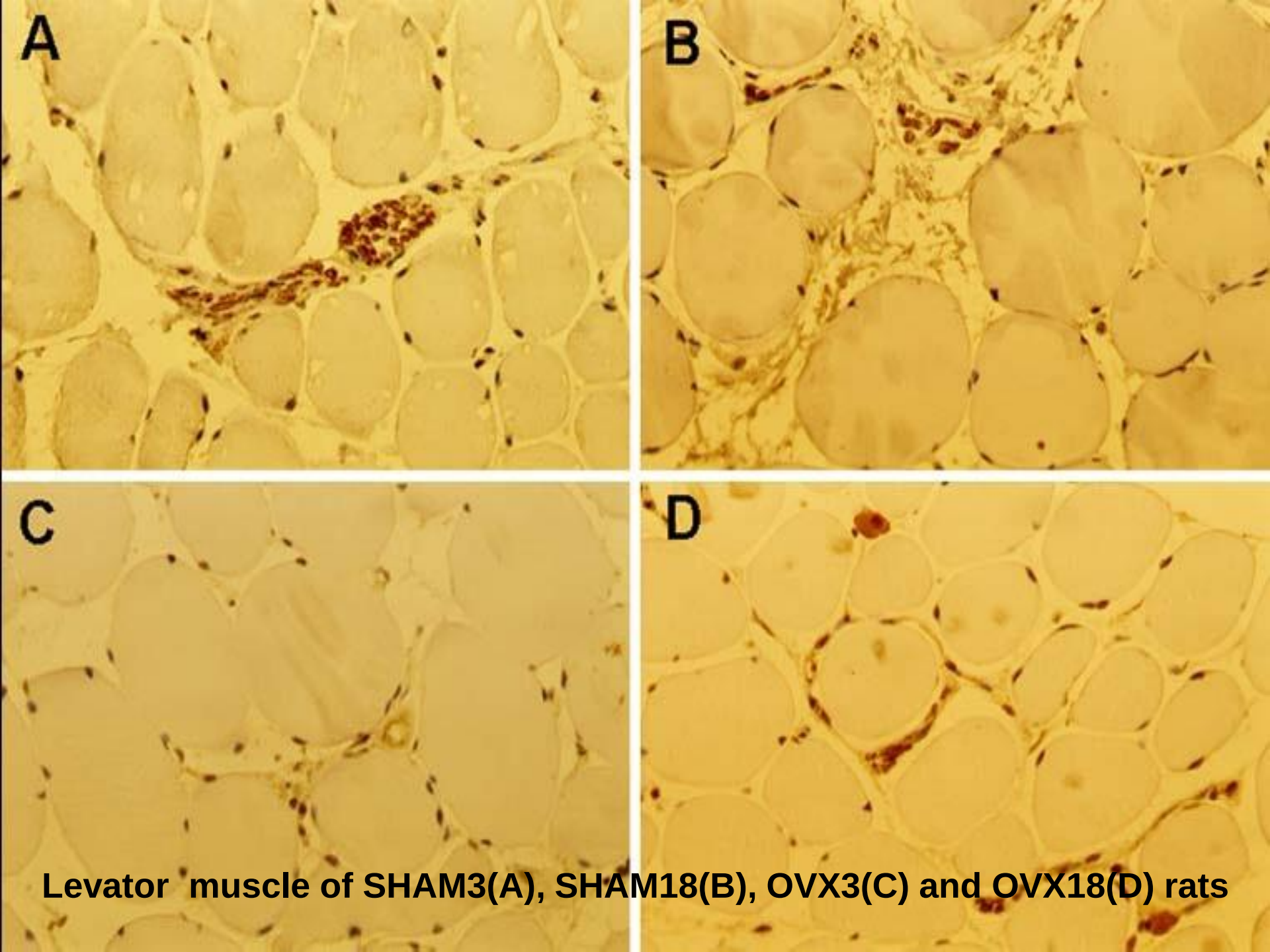
Results- V

- Ghrelin receptors were demonstrated immuno-histochemically in the urethral and anal canal submucosa, in both young and adult animals, suggesting a direct receptor-mediated effect.

- In the striated pelvic floor muscles, ghrelin receptors were NOT demonstrated immno-histochemically in both groups suggesting that ghrelin produced its positive effect via ? stimulation of GH release.



Urethral submucosa of OVX3 (a) and OVX18 (b) and anal canal submucosa of OVX3 (c) and OVX18 (d) rats



Levator muscle of SHAM3(A), SHAM18(B), OVX3(C) and OVX18(D) rats

Results- VI

- Serum growth hormone levels were significantly lower in SHAM 18 than in SHAM 3 animals.

- OVX significantly decreased growth hormone levels further in OVX18 rats.

- Ghrelin and combined estrogen/ghrelin administration significantly increased growth hormone levels in OVX18 rats with a greater increase after combined administration.

Results- Summary

- The natural increase in expression of ageing markers in the urethra, anal canal and striated pelvic floor muscles of OLD Fisher 344 rats is increased FURTHER after OVX compared to young adult animals.

- OVX and subsequent estrogen deprivation EXACERBATES inherent ageing alterations in the pelvic floor apparatus of OLD rats.

- In YOUNG rats, estrogen administration reversed the OVX-induced adverse changes.

Results-Summary

- In OLD rats, ONLY COMBINED estrogen and ghrelin administration COMPLETELY abolished the OVX-induced adverse changes but neither ghrelin and/or estrogen separately produced a similar effect.

- Ghrelin exerts a positive effect on all pelvic floor ageing markers in old rats either by itself through ghrelin receptors or via stimulating GH release.

- The differential effect on different target tissues may be due to different estrogen/ghrelin receptor density.

Conclusions

- There is a synergistic and DELETERIOUS interaction between hypoestrogenism and normative ageing on the pelvic floor of old OVX rats.

- **The proof of concept of independent and non estrogen-mediated ageing changes in the pelvic floor and the ability of estrogen/ghrelin combination to reverse these changes was achieved in our animal model with these data.**

- Our results should be examined in clinical studies to investigate the full therapeutic potential of estrogen/ ghrelin combination in geripausal women.

Possible Clinical Applications

- Prevention of geripausal pelvic floor disorders in women at risk (window hypothesis).
- Explain the relationship between obesity and geripausal pelvic floor disorders (ghrelin regulates food intake and satiety).
- Local therapy of geripausal SUI (or FI) similar to bulking agents.

Work in Progress

- Adjuvant peri-operative treatment with repair of geripausal pelvic floor disorders (particularly tape and mesh repair) to improve wound healing and prevent erosion- ***[work with Jan Deprest and Dirk de Ridder- IUGA 2012 Grant].***

- Potential role in OAB in geripausal women (ghrelin receptors were identified in the human bladder, ghrelin inhibited KCl-induced contractions of rat detrusor muscle strips)- ***[work with Michael Chancellor- personal communication].***

- Electron microscopy studies of the number of satellite cells/muscle cell in the striated pelvic floor muscles.

Key Messages

- Ageing of the female pelvic floor is similar to cognitive function that gradually declines with ageing but is accelerated after menopause.

- Geripausal pelvic floor dysfunction is definitely expected to increase because the female population of the world is ageing **(Demand for health care service is expected to increase by twice in the next decade).**

- A woman's programmed biological age or reproductive span CANNOT be reversed but medical science can substantially contribute to geripausal pelvic floor research.

Acknowledgment

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1- Rizk DEE, et al. Estrogen and ghrelin increase number of submucosal urethral and anal canal blood vessels in ovariectomized rats. Urology 2005; 66: 1343-48.

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