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SUPPLEMENTARY MATERIAL

corresponding to:

Ontogenetic consequences of dysgenic crosses in *Drosophila virilis*

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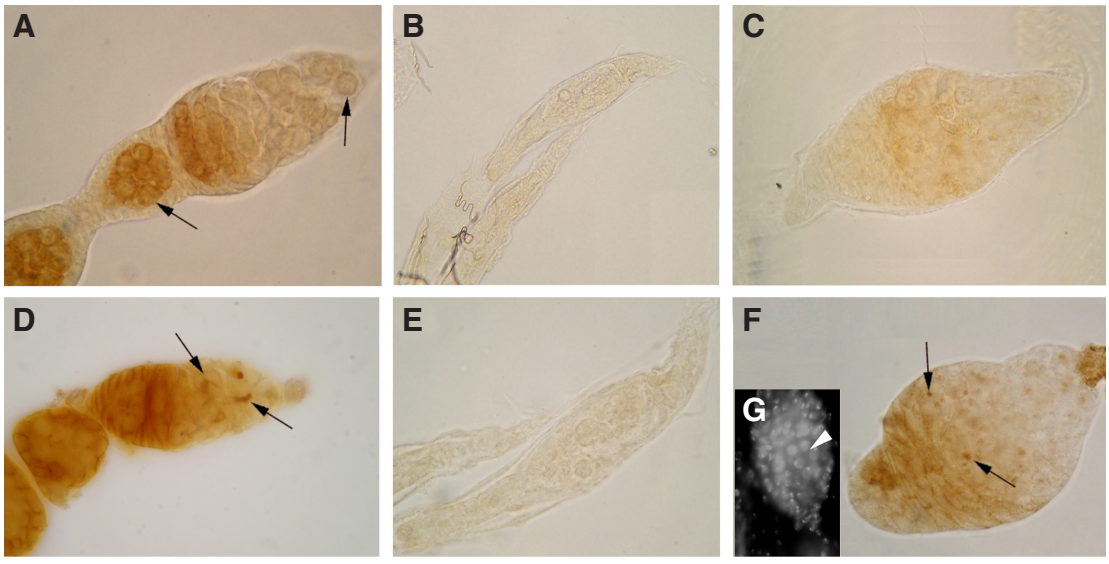
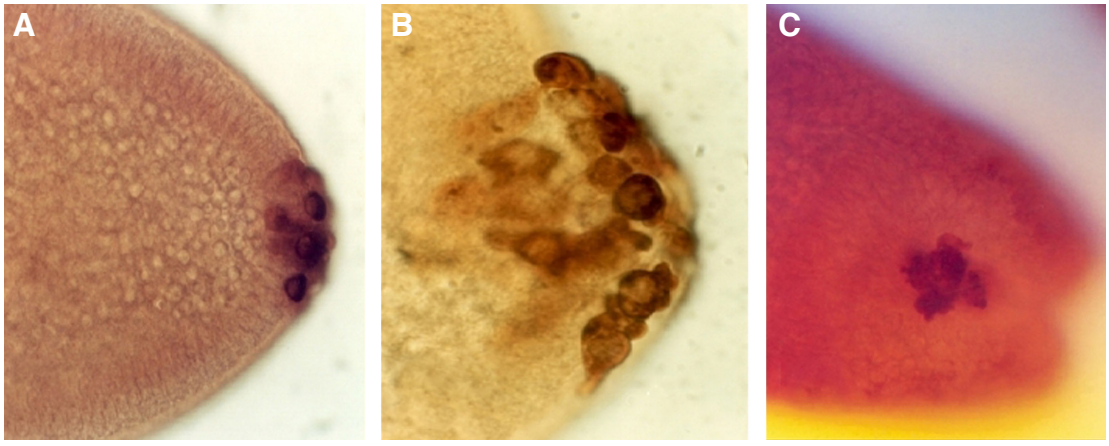
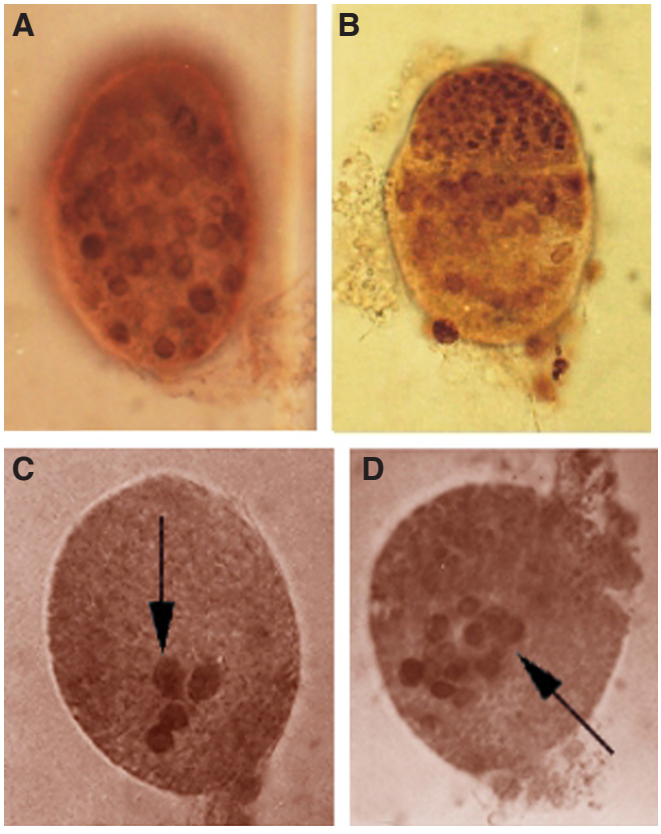
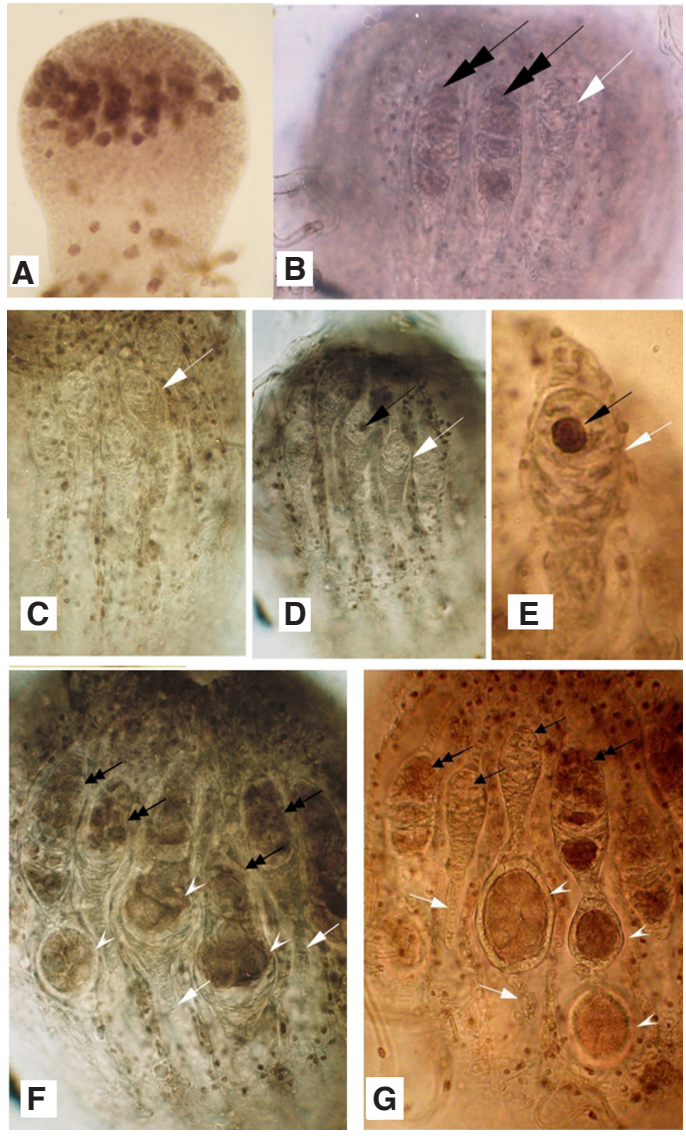


Supplementary Fig. S1. *Drosophila virilis* ovarioles from strain 9 (control) and dysgenic females stained with anti-Vasa antibodies (A-C), 1B1 antibodies (D-F) and DAPI (G). (A) *normal ovarioles with stem oogonial cells and follicles indicated by arrows. Vasa staining is prominent in the germ line cells and trophocytes cytoplasm;* **(B**,**C)** *Typical agametic and tumor-like ovarioles which are not stained by Vasa-antibodies and apparently do not contain germline cells.* **(D)** *Phenotypically normal ovariole with typical fusomes indicated by arrows stained by 1B1 anti-bodies in the germ line cysts.* **(E)** *Agametic ovariole not stained by 1B1 antibody and apparently lacking any germline cells.* **(F)** *Tumor-like ovariole containing multiple undifferentiated cytocysts. Arrows indicate presumptive spectrosomes in the undifferentiated cytocystes.* **(G)** *Tumor-like ovariole stained with DAPI; the white arrowhead indicates large undifferentiated cystocyte. 40x.*



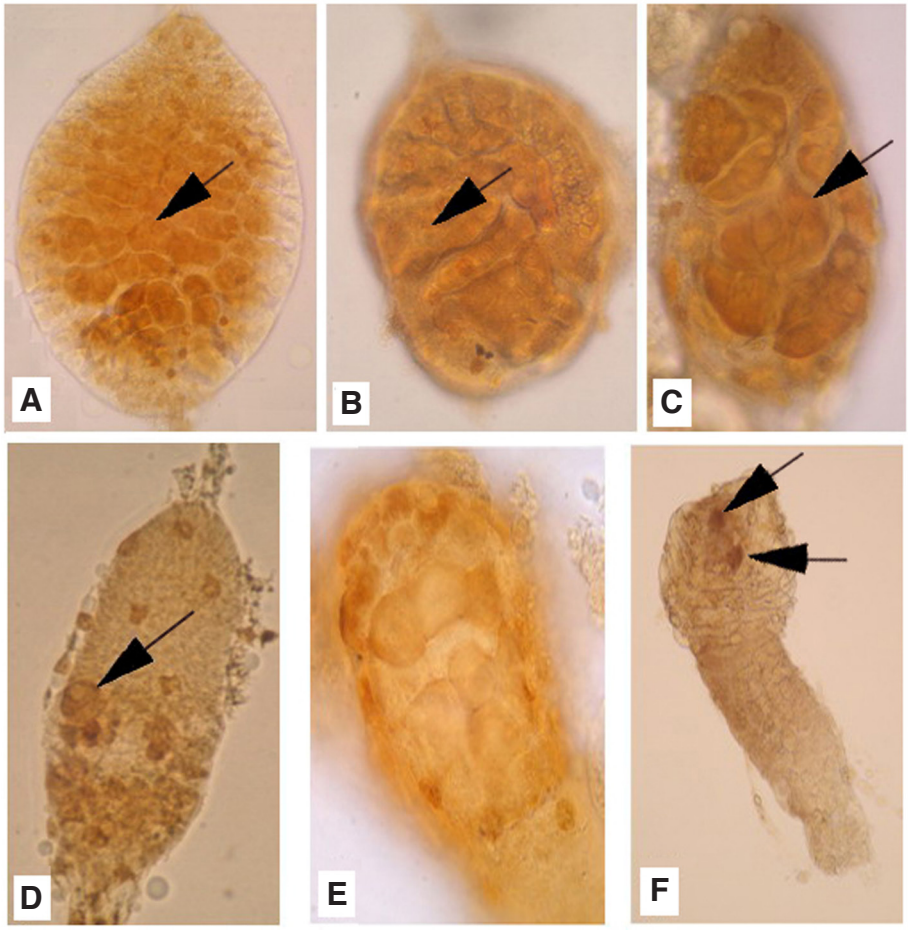
Supplementary Fig. S2. Pole cells in *Drosophila virilis* early embryos were stained with anti-Vasa antibodies. (A) *Normal-appearing pole cells (brown) are shown at the posterior end of the dysgenic embryo at the early blastula stage.* **(B,C)** *Moving pole cells with well-developed filapodia are seen in dysgenic embryo.The stages are as defined by Campos-Ortega and Hartenstein (1985). Magnifications:* **(A)** *60x,* **(B)** *-100x oil immersion;* **(C)** *40x.*





Supplementary Fig. S3. (Left) ***Drosophila virilis*****ovaries of dysgenic larvae were stained with anti-Vasa antibodies. (A,B)** *Gonads are shown exhibiting the normal appearing process of ovariole formation from germ cell proliferation* **(A)** *to the formation of filaments of primordial ovarioles at the top of a gonad* **(B)**. **(C,D)** *Different examples of abnormal localization and quantity of germ cells in the ovary are shown. The arrows indicate individual stained large germ cells; 40x.*

Supplementary Fig. S4. (Right) ***Drosophila virilis*****ovaries of dysgenic pupae stained with anti-Vasa antibodies. (A,B)** *The beginning of ovariole formation is shown in early pupae (black double-headed arrows).* **(C-E)** *Dysgenic ovaries in late pupae.* **(C)** *Sterile agameticovarioles are shown.* **(D)** *One ovariole has one stained oogoinial cell (black arrow); 40x.* **(E)** *The same sterile ovariole with a stained large oogonial cell is shown; 100x.* **(F,G)** *The formation of the pseudo tumor-like ovarioles in dysgenic late pupae. White arrowheads indicate follicles; white arrows indicate pedicils. Black double-headed arrows indicate the germarium zones. All images represent the situation when in the same ovary the individual ovarioles comprising the ovary are at different developmental stages altogether lacking or containing one or two normal-looking follicles; 40x.*



Supplementary Fig. S5. ***Drosophila virilis*** **testes of third instar dysgenic larvae (A-E) and early pupae (F) were stained with anti-Vasa antibodies.** **(A)** *The normal orientation and structure of spermatid clusters (black arrow) in normal gonads is shown at 100x.* **(B,C)** *The testes with severely disturbed spermatid cluster orientation, indicated by arrows; 100x.* **(D,E)** *The abnormal testes apparently lacking the spermatid clusters altogether; the black arrow in* **(D)** *indicates a large stained cell of unknown nature.* **(F)** *Small, abnormal, pre-pupae testis containing only two germ cells indicated by arrows lacking normal cluster organization; 40x.*

SUPPLEMENTARY TABLE S1

PRIMERS USED FOR PCR

	Sequence	Application
1	5'-AGGAATGCCTAGCCGCCAAA-3'	*Ulysses* forward and reverse primers
2	5'-AACGCTTGCAGTTCGAGGGA-3'	for RT-PCR
3	5'ACGGTGAGGAGCTAGTGCAAACAA-3'	*Penelope* forward and reverse
4	5'-TTCGTGTCTGTTCCACTGTGTCCA-3'	primers for RT-PCR
5	5'-ACACGTTGGCGGAATGCGAAA-3'	*Penelope* forward and reverse
6	5'-TGAGTGTGGCAGTTGGCGATG-3'	primers for RT-PCR
7	5'-ACGGACCCAGCAAAGTTTGGAGAA-3'	*Paris* forward and reverse primers
8	5'-AGCTCACCAACACCTTTCGACGAT-3'	for RT-PCR
9	5'-TGGCTCTATGGAGTGCAGATTTGG-3'	*Helena* forward and reverse primers
10	5'-TCGACTGTGTGCACTTTGAGGTCT-3'	for RT-PCR
11	5'-TTACGGTTCCAACAAGCGCACC-3'	*Rp49* forward and reverse primers
12	5'-GCGCTCAACAATCTCCTTGCGT-3'	for RT-PCR
13	5'-GTTGGAATTTATTAATTGTTCTTTTCG-3	*Penelope* primers revealing intronic
14	5'-GTAACAATCTCCACGTCAAAAAGCG-5'	and intronles copies

q-helena-f: q-helena-r: dvir_rp49-f: dvir_rp49-r:

SUPPLEMENTARY TABLE S2

THE INFLUENCE OF PARENT AGE ON DYSGENESIS FREQUENCY (%)

Dysgenic cross (strain 9 females X strain 160 males)

Parent age / F1 progeny age	1-5 days	6-10 days	11-15 days	16-20 days
♀♀ 7 days old	70,2± 5,7	58,3± 3,9	49,8± 4,2	53,2± 3,7
♀♀ 35 days old	66,1± 4,6	64,5± 3,8	60,4± 4,0	55,5± 5,0
♂♂ 10 days old	45,5± 4,8	37,7± 2,9	27,3± 3,0	16,8± 2,3

Reciprocal cross (strain 160 females X strain 9 males)

Parent age / F1 progeny age	1-5 days	6-10 days	11-15 days	16-20 days
♀♀ 7 days old	1,8± 1,7	2,8± 2,8	2,2± 2,2	0,5± 1,1
♀♀ 35 days old	0	1± 1,5	0,5± 1,0	0
♂♂ 10 days old	2,0± 1,5	2,2± 1,9	1,6± 1,5	1,6± 1,5

SUPPLEMENTARY TABLE S3

THE INFLUENCE OF GROWING TEMPERATURE ON GONADAL STERILITY IN PARENTAL STRAINS AND DYSGENIC HYBRIDS

strain 9			
Flies with gonadal atrophy in %	18°	25°	28°
♀♀	2,3±0,7	0,9±0,7	0,94±0,7
♂♂	3,6±0,7	0,9±0,6	4,17±0,8
strain 160			
Flies with gonadal atrophy in %	18°	25°	28°
♀♀	12,88±0,6	11,83±0,7	9,02±0,7
♂♂	5,7±0,3	6,6±0,3	6,09±0,4
Dysgenic hybrids			
Flies with gonadal atrophy in %	18°	25°	28°
♀♀	89,8±0,2	53,2±0,2	55,03±0,5
♂♂	80,4±0,3	57,1±0,4	60,39±,05