CENP-V expression in human eggs and CENP-V depletion in *Caenorhabditis elegans*

Master's Degree in Advanced Biology: Research and Application

University of Seville, 2023

Master's Thesis; Scientific Article Format
Format of the scientific journal indexed in SCI, *Nature Communications*.
Master's Thesis Tutor: Mariola Chacón Rodríguez
Author: **Carmen María Vargas Fernández**

Abstract

CENP- V is a kinetochore protein needed for meiosis progression in mice oocytes. Cenp-V -/- mice oocytes arrest at metaphase I or proceed to metaphase II harbouring lagging chromosomes. The goal of this study is in one hand to study CENP-V directly into the human egg and in parallel to study CENP-V in a model system in which oocyte availability is unlimited and meiotic prophase can be analysed. As a first step, we optimised the immunofluorescence of CENP-V in human oocytes from discard material of *in vitro* fertilisation treatment. We found that the pellucid zone needs to be removed to allow the penetrance of the antibody. Next, we have established a loss-of-function assay by RNAi in an alternative model, *Caenorhabditis elegans*. We find that the depletion of F25B4.8, the ortholog of CENP-V in the nematode causes chromosomes aberrations. Hence, we conclude that *C.elegans* can be used as a model to study the role of CENP-V during oocyte meiosis and thus to complement the analysis in the human egg. Moreover, the ortholog of *Cenp-V* has been never studied in the nematode before.

Introduction

William Earnshaw, in 2008, described the human CENP-V for the first time as a kinetochore component in proteome analysis of human mitotic chromosomes. Human CENP-V is formed by 275 amino acids and 29.7 kDa of molecular weight. In humans CENP-V is located on chromosome 17p11.2. The proline-rich region of human CENP-V allows a correct association with the SH3 domain of Src family kinases (SFKs) and activates them. Another highly cysteine array of CENP-V is able to associate with stabilised microtubules (MTs). These facts confer CENP-V a role in directional cell migration. CENP-V also supports tubulin acetylation through basal bodies and axonemes of primary cilia. CENP-V is highly conserved among vertebrates and has a conserved domain annotated as glutathione-dependent formaldehyde-activating enzyme (Gfa), a putative DNA binding domain conserved in the nematode *Caenorhabditis elegans*[1, 2].

In HeLa cells, CENP-V is a kinetochore protein needed for the progression of mitosis. CENP-V is located to the mitotic kinetochores from prometaphase to metaphase. CENP-V coordinates chromatin condensation, positionates sister chromatid centromeres and targets chromosome passenger complex (CPC). The CPC is implicated in the attachment of spindle MTs to the kinetochores. On the one hand, depletion of CENP- V in HeLa cells cause impaired metaphase alignment on chromosomes and lagging chromosomes during anaphase. CENP-V is needed for the normal structure of the inner centromere in mitotic chromosomes as its depletion drives to several changes in the distribution of centromeric components and CPC components. CENP-V depletion causes cell death in HeLa cells. On the other hand, overexpression of this protein causes hypercondensation of pericentromeric heterochromatin or interfere with its decondensation during mitotic exit. Moreover, this overexpression is highly toxic, dying half of the cells in interphase. It is suggested that CENP-V overexpression causes a huge disruption in cell cycle progression during S or G2 phases [1].

During meiosis Nabi, D. et al. demonstrated in Cenp-V -/- mice that oocytes arrest at metaphase I and chromosomes captured by microtubules is impaired leading to aneuploid eggs. During meiotic prophase, CENP-V was found in the vicinity of centromeres and the

surrounding heterochromatin co-staining with the centromere marker ACA. At meiosis resumption, it is observed three different pools of CENP-V: at the chromosomes, at the microtubule organising centres (MTOCs) and the meiotic spindles. They suggested that during female meiosis CENP-V is located from the centromere region in prophase to along metaphase I chromosomes, to the MTOCs, and the microtubules during metaphase I and polar body extrusion (PBE). In the absence of CENP-V aberrant spindle shapes are observed. In addition, *in vitro* Total Internal Reflection Fluorescence (TIRF) microscopy showed that CENP-V binds, diffuses and bundles microtubules. These experiments concluded that CENP-V has an important role in kinetochore-microtubule attachments, and it is necessary for proper chromosome alignment and spindle formation. In summary, CENP-V contributes to the progression of mammalian meiosis. In spermatids CENP-V was not found in the sex body. Surprisingly, they did not find any failure in meiosis prophase in *Cenp-V* $^{-/-}$ spermatids [3].

Nowadays, parenthood is delayed. In Spain in 2018, almost 80% of women between 25-29 years old had not yet conceived the first child and more than 50% had it after 35 years old. In Europe the average of women having children after 40´s has been duplicated from 2001 to 2019 [4, 5]. Arrest period of oocytes is prolonged, generating a higher probability of aneuploidy and the ovarian age is the main cause of infertility. The oocyte quantity and quality are directly associated with reproductive ageing. Thus, only approximately 350 oocytes will escape cell death and be ovulated during the human reproductive life span, corresponding to <0.006% of the 6–7 million potential eggs initially formed during fetal development. In the mid-thirties the fertility decreases until menopause begins [6, 7]. Due to this situation, more women are requiring assisted reproduction treatment. Female meiosis in humans is a long process. Already in foetal state, oocytes enter the first stage of meiosis, the prophase I. In this stage, homologous chromosomes are linked as one pair, known as bivalent chromosomes, requiring the induction of DNA double-strand breaks (DSBs). At birth all oocytes are arrested at the dictyate stage until puberty begins. At puberty one or few oocytes resume the meiosis in a one month cycle every month until menopause. Also, approximately 80% of the oocytes that enter meiosis are culled by birth and less than half of those remaining survive through puberty [7]. From the Germinal Vesicle stage (GV) oocyte undergoes nuclear envelope breakdown (NEBD) followed by the first meiotic division. During NEBD, the chromosomes are fully condensed. At metaphase I, the bivalents are aligned in the centre of the spindle. The extrusion of the first polar body (PB) with half of the homologous chromosomes occurs during anaphase I. The mature oocyte is arrested again at metaphase II. The ovulation happens and the oocyte migrates down the fallopian tube. Anaphase II takes place when the oocyte is fertilised. At this stage, sister chromatids are segregated by the second meiotic spindle and the second polar body extrusion takes place. If fertilisation does not take place, menstruation starts [8-13]. While the oocytes stay arrested in the ovary, the probability of suffering errors increases, and it is the same with advancing age. The period of time which the ovary keeps the oocytes is more time gained to errors in chromosome segregation. Women younger than 35 years present a basal rate of errors close to 20%. In older women this rate could be as high as 60% [12-14]. The proposed causes of these errors' incrementation are: the gradual deterioration of cohesin, damage in the telomeres while advancing ageing, causing chromosome end fusions generating aberrant chromosome segregation, and erroneous attachment of microtubules to the

kinetochores causing mis-segregation if SAC does not correct the error during arrest. Cohesin maintains sister chromatid cohesion throughout oocyte lifespan and it is responsible to enter chromosomes' oocytes into meiosis. The loss of cohesion is related with the loss of chiasmata in meiosis I and sister centromere's weakness at anaphase II. These factors are considered age-dependent oocyte chromosome mis-segregation. A well-balanced control system is needed to avoid chromosomes' mistakes. However, this system does not work perfectly all time long [3]

In mammals, female meiotic prophase occurs during embryogenesis making the analysis almost impossible. Hence, many studies have been performed in the nematode *Caenorhabditis elegans*, a model system that allows molecular studies. *C.elegans* has two forms of life: female hermaphrodites (XX) and male worms (genetically X0). The dominant form is the female hermaphrodite one. This hermaphrodite worm produces sperm and oocytes, and the male worms produce sperm only. *C.elegans* adult hermaphrodite contains two U-shaped (also called S-shape) gonads and one common uterus. Within the same animal, the gonad shows every stage of the meiotic prophase. At the most distal tip, also called the proliferative zone, there are germ cells nuclei under mitotic division and thereafter enter meiotic prophase I, where are the five sequential stages: leptotene, zygotene, pachytene, diplotene and diakinesis. At leptotene and zygotene chromosomes start to pair and align and DSB are formed and in the early pachytene stage, the chromosomes synapse. The synapsis of the homologous chromosomes occurs thanks to the assembly of synaptonemal complex (SC). Once the oocytes enter into the mid pachytene stage, the synapsis is completed and the chromosomes are fully shaped. SC ensures a proximity close enough to generate a crossover by double strand break (DSB)-induced recombination. This phenomenon manifests as chiasmata and it is crucial because chiasmata orient homologous chromosomes toward opposite spindle poles. At diplotene, the SC starts to disassemble and the chromosome condensation begins. For the first time, chiasmata can be seen holding homologous chromosomes. Finally, at diakinesis, the six distinct DAPI-bodies or bivalent (the six pairs of homologous chromosomes) are observed together by chiasmata. The spermatheca is found right after diakinesis is over. Once the oocytes are fertilised, the bivalents line up on the spindle during the subsequent metaphase I. During anaphase I, the two duplicated homologs are separated to opposite poles of the spindle generating two cells. These cells are not gametes yet. Cells need to pass through a second division in which sister chromatids are segregated to form haploid gametes. All this important processes in meiosis are conserved between the nematode and mammals [15-17].

Nabi, D. et al. described that CENP-V protein has an important aneuploidy factor in oocyte meiosis in young and aged mice females. The important role of CENP-V observed in oocyte meiosis in the murine model and the differences regarding function and localisation compared to mitosis in HeLa cells arise the question whether CENP-V could play other roles during meiosis in human eggs and lead to the need to do translational research in the human female meiosis. However human eggs are limited; to overcome this limitation and an alternative model system in which the role of CENP-V in meiosis can be easily studied to complement the analysis in human egg is therefore mandatory. CENP-V is evolutionarily conserved, BLAST searches revealed proteins related to CENP-V among plants and humans [3, 18]. F25B4.8 is the ortholog of CENP-V in the nematode *C.elegans* and has never been characterised. Thus, the two main objectives of this study are (1) to optimise the

immunofluorescence essay of CENP-V in human oocytes and (2) to study the depletion of CENP-V in *Caenorhabditis elegans*.

Results

CENP- V distribution in immature human eggs by immunofluorescence staining.

In order to optimise the immunoassay of CENP-V in human oocytes we performed two different protocols using discard material from *in vitro* fertilisation treatments (Fig. 1). Three different CENP-V commercial antibodies have been tested in unmatured human oocytes (see supplementary table 1). The main difference of both protocols lay on the presence or absence of the pellucid zone. The pellucid zone is a delicate network of thin interconnected filaments.

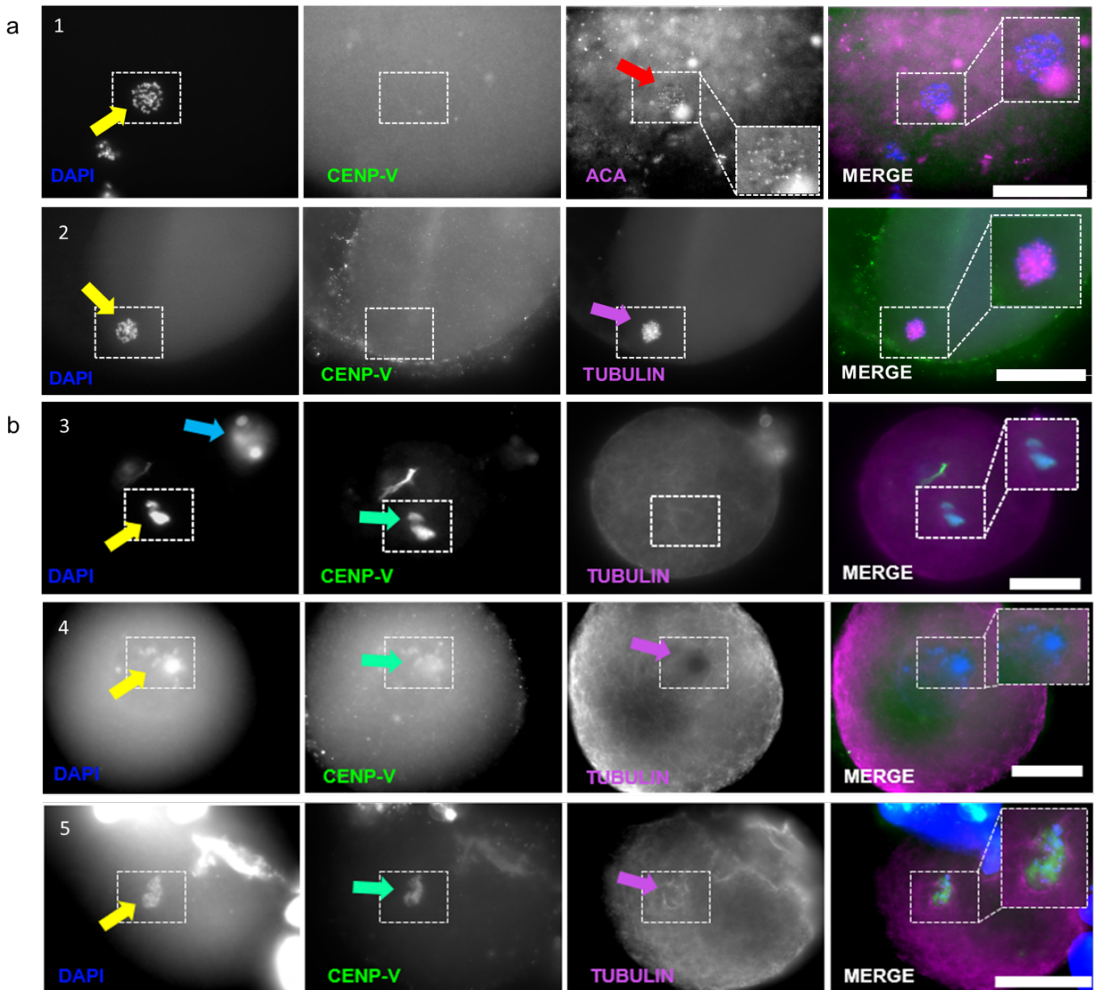

Fig. 1 CENP-V immunofluorescence of human oocytes. (a) Examples of oocyte's immunofluorescence with pellucid zone (1 and 2). **(b)** Examples of oocyte's immunofluorescence without pellucid zone (3, 4 and 5). Blue arrow indicates the presence of granulosa cells. Yellow arrow indicates DNA. Green arrow indicates the presence of CENP-V. Red arrow indicates the presence of ACA. Magenta arrow indicates the presence of tubulin. Scale bar = 50 µm. DNA was stained by DAPI (blue); CENP-V was stained with anti rabbit CENP-V polyclonal (1, 3-5) or mouse anti-CENP-V monoclonal (2) (green); centromeres were stained with anti human ACA (1, magenta) and Tubulin was stained with anti rat α-tubulin (2,

It is primarily composed of four glycoproteins: ZP1, ZP2, ZP3, and ZP4 [19]. This structure affects the penetration of the CENP-V antibody in the cells. Thus, some cells were treated with tyrode's solution to remove the pellucid zone. We have tried two batches of the anti-rabbit CENP-V polyclonal. The CENP-V of the Fig. 1b, picture 3 have been stained with a different batch from Fig. 1a, picture 1, Fig. 1b, picture 4 and Fig. 1b, picture 5. It seems to be that the batch used for Fig. 1b, picture 3 presented more fluorescent signal than the other batch. As we expected, the CENP-V of oocytes with pellucid zone did not stain (Fig. 1a) suggesting that the composition of pellucid zone did not allow the entrance of CENP-V antibody. However, in those oocytes in which the pellucid zone was removed (Fig. 1b), CENP-V was successfully stained. Due to this difference, we strongly believe that remove pellucid zone is a crucial step to obtain CENP-V staining by immunofluorescence (IF). Indeed, in those IF experiments maintaining the pellucid zone CENP-V did not colocalize either with DAPI neither with the centromeric marker ACA or tubulin signal (Fig. 1a, pictures 1,2). Tubulin signal is observed the perimeter of GV and in early spindle depending on the maturation stage of the oocyte (Fig. 1a, picture 2).

F25B4.8 depletion in *C.elegans* leads to chromosome aberrations in oocytes at diakinesis. The putative ortholog of CENP-V is the F25B4.8 and the sequence of the epitope used to produce the commercial antibody does not fully align with the human epitope (Supplementary Fig. 1) thus we did not expect much success using this antibody in the nematode. Hence, we decided to perform first the depletion of F25B4.8 to study the role of CENP-V during meiotic prophase in oocytes. We performed loss-of-function experiments by RNAi feeding using to the target gene; in this case F25B4.8, the putative ortholog of CENP-V [20]. As the previous work in murine oocytes suggest that the absence of CENP-V leads to aneuploid embryos, we quantified the number of homologous chromosome pairs at the diakinesis stage right before the oocytes enter in the spermatheca in the absence of CENP-V. DNA from control and F25B4.8 depleted worms was stained with DAPI and DAPI-bodies were counted at diakinesis through a z stack every 200 nm (Fig. 2, Supplementary Fig. 2 and Fig. 3). The last four oocytes at diakinesis are the ones that were quantified to avoid oocytes from late diplotene [21]. A three-dimensional stack through single oocytes at diakinesis was carried out to see the effect of HT115(DE3) bacteria transformed with the L4440 vector containing a fragment corresponding F25B4.8 depletion (Fig. 3). As expected, almost 90 % of the control showed six pairs of homologous chromosomes (bivalents) in diakinesis. Just a few oocytes showed variations of numbers of DAPI-bodies like 5 or 7 pairs which we assume are errors of the quantification through the z-stack. Although we cannot discard a possible side effect of the control vector. However, CENP-V depletion showed a huge variation in the number of DAPI-bodies with roughly 76% of the cells showing higher or lower number than the six expected bivalents (Fig. 3a). The population of oocytes in the F25B4.8 depleted gonad showed a high variability in the DAPI bodied number compared to the control gonad (Fig. 3b). In control conditions we did not find any irregular phenotype and regular and compacted shapes were observed in the control gonad compared to the F25B4.8 depleted cells in which we observed chromosome fusions, fragmentations and complete separate bivalents (Fig. 3c). F25B4.8 depleted oocytes (Fig. 3c) show chromosomes fusions and fragmentations (Fig. 3c, panels 3,4,5,8) decompacted structures (Fig. 3c, panel 7), and complete separated bivalents (Fig. 3c, panel 6).

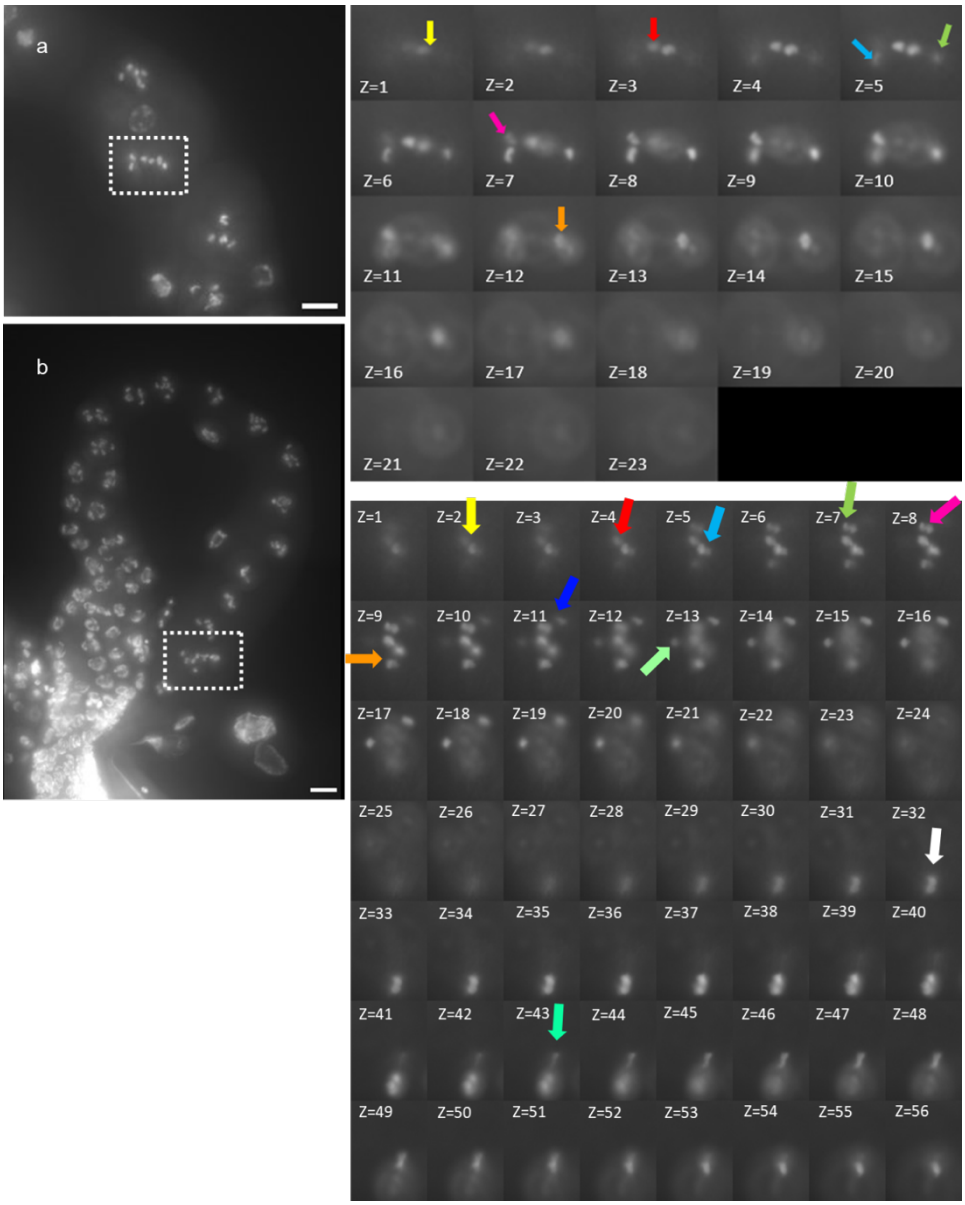

Fig. 2 Example of the image analysis at diakinesis in C.*elegans*. a Diakinesis zone of the control gonad (left). On the right an example of the six expected pairs of homologous chromosomes (bivalents) through the z-stack stained by DAPI. In this case, 23 planes (z=1 to z=23) were significant to analyse the oocyte out of 28. **b** Diakinesis zone of the F25B4.8 depleted gonad (left). On the right an example of the ten pairs of homologous chromosomes observed through the z-stack stained by DAPI. In this case, 56 planes (z=1 to z=56) were significant to analyse the oocyte out of 56. Each colour arrow indicated the presence of a new DAPI body. Dashed line indicated the selected oocyte. Scale bar= 10 µm

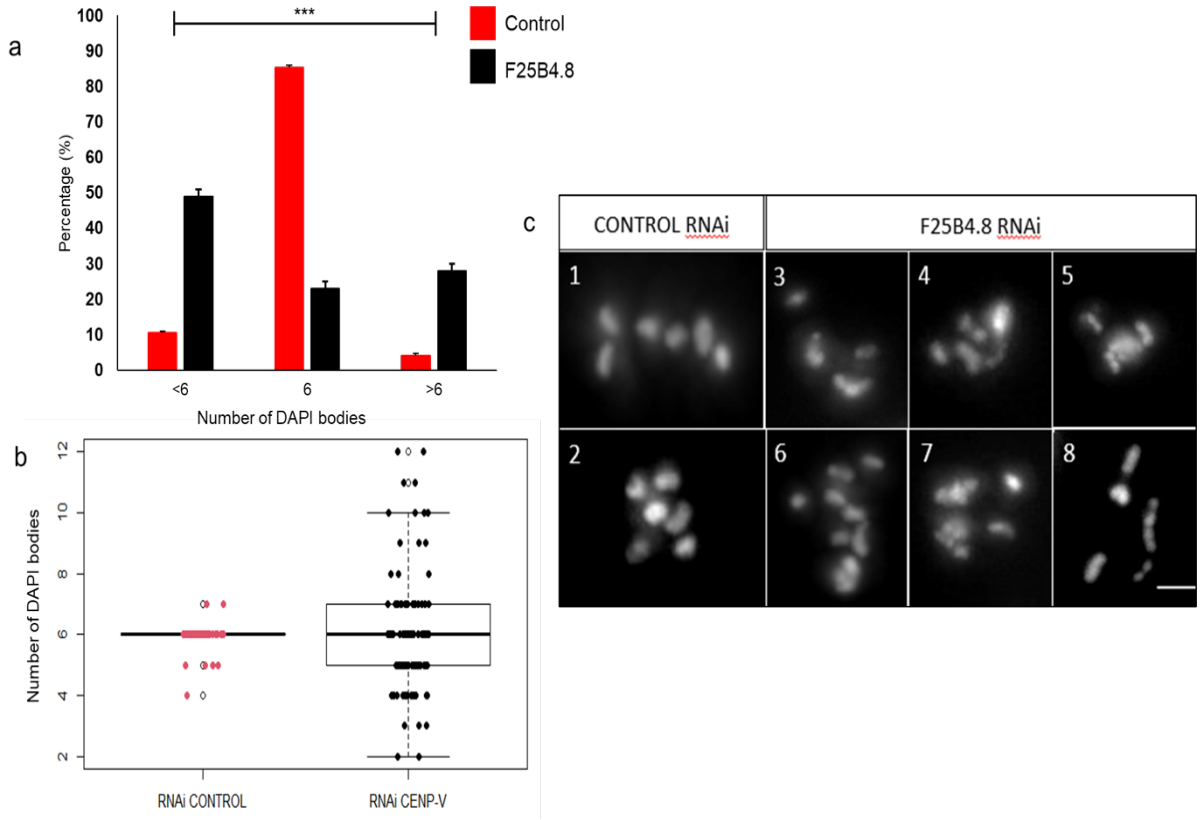

Fig. 3 Number of DAPI stained bodies at diakinesis in *C.elegans*. (a) Percentage of cells observed at diakinesis in *C.elegans* versus number of DAPI bodies in control (red) and CENP-V depleted (black) oocytes. **(b)** Box plot of DAPI bodies in control (red) and CENP-V depleted (black) oocytes. Middle line is the median, the lower and upper boxes correspond to the first and third quartiles and outlying points are plotted beyond the end of the whiskers; no coloured dots are the average of the outliers (**c)** Representative maximum projections of a three-dimensional stack through a single oocyte at diakinesis. Note that control shows the 6 expected pairs of homologous chromosomes in diakinesis (1 and 2). Compared to the F25B4.8 depleted oocytes (3 to 8). DNA was stained by DAPI (grey). Scale bar= 5 µm. n= 152 cells from 5 independent experiments.

All together these experiments suggest that the depletion of the CENP-V ortholog will result likely in aneuploid oocytes.

Analysis of the synaptonemal complex in the F25B4.8 depleted gonad of *C.elegans*.

In the mice spermatids in pachytene CENP-V distributes along the chromosomes except in the sex body, the only chromosome pair that remains unsynapsed [3]. We hypothesized that CENP-V could be involved in chromosome synapsis. To test this hypothesis, we performed immunofluorescence assay against SYP1 in the gonads of control and F25B4.8 RNAi depleted in the oocyte of *C.elegans* (Fig. 4). SYP-1 and SYP-2 are proteins of the central region of the SC in *C.elegans*. SYP1 localises at the interface between intimately paired, lengthwise-aligned pachytene homologs [22], thus six strings are expected to be observed. This experiment has been only once performed and therefore an extended analysis must be done in the future. However, in this preliminary analysis we observed that in the absence of CENP-V some

chromosomes in pachytene seems to be unsynapses as some cells show many SYP1 strings, as depicted in Figure 4.

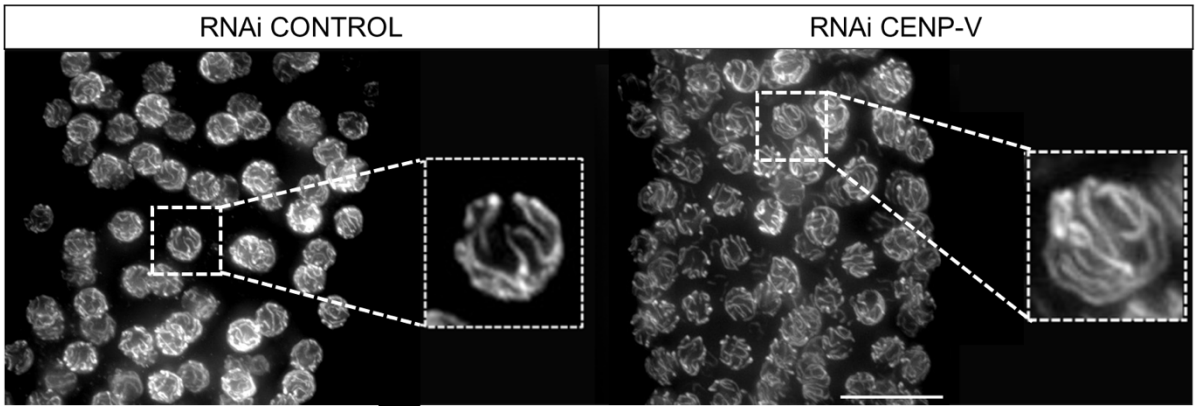

Fig. 4 Control and CENP-V depleted pachytenee nuclei stained with an antibody against the SYP-1. The images shown are projections approximately halfway through 3D data stacks that encompass entire nuclei. Scale bar= 10 µm

Discussion

The current study uses a dual approach to study CENP-V in meiosis. We have strong evidence to believe that CENP-V is a crucial protein for chromosome segregation and aneuploidy in young and aged oocytes. CENP-V shows a different localization between mice and humans, which leads to the assumption that the protein plays a different role in the human oocyte meiosis. Therefore, the first approach is to study CENP-V directly into human eggs. However, the access to human eggs is limited to the discard material from *in vitro* fertilization treatment. Hence the second approach is to study CENP-V in *C.elegans* as a parallel model system. In comparison to other models *C.elegans* allows to follow all meiosis stages within the same animal and CENP-V has been never analysed before. Furthermore, *C.elegans* has holocentric chromosomes, which are less characterized than the monocentric type. Consequently, any insight into CENP-V in *C.elegans* that goes beyond its role in meiosis will be of general interest. This work has been carried out in the lab of Dr. Chacón, recently established at the Sevilla University. The idea is that both studies will complement each other. Thus, the goal of this "TFM" is to optimise the immunofluorescence assay of CENP-V in the human oocytes coming from the clinic and to test *C.elegans* as a model system to decipher the role of this protein during meiotic prophase. In the long term, Chacón's lab will use the nematode to elucidate basic mechanisms and use human oocytes to test the clinical significance of the findings in the worm.

During these two months we received cells from the patients 7 times. First, we optimized the workflow between the clinic and the lab. The DAPI staining of the GV oocytes indicates that the time between oocyte retrieval and fixation is crucial for cell survival. Thus, many oocytes were death or unhealthy before fixation (data not shown). Tyrod's acid treatment is very harmful for the cells but unfortunately it seems to be the only way for some antibodies to enter in the cell. Thus, we conclude that the transfer of the oocytes from the clinic to the lab

must be optimized and only a mild Tyrod's acid treatment can be performed. This was the only scenario in which CENP-V protein could be observed in the nucleus colocalizing to the DAPI staining. In the particular case of the antibody against CENP-V we noticed differences between monoclonal and polyclonal antibodies. Only a commercial polyclonal antibody gave positive results and trustable signal using DAPI staining as a reference for the DNA. Moreover, two different batches of commercial polyclonal antibodies have been used and differences among them has been observed. In a study of mice post meiotic spermatids they observed CENP-V only at the pericentromeric heterochromatin however we observed CENP-V covering the entire DAPI signal. In this study they used a home-made rabbit antibody against CENP-V [23]. We cannot discard the difference between antibodies to be the reason for the different pattern. Thus, it might be worth being to test the home-made antibody against CENP-V in the human egg in the future. CENP-V is conserved among eukaryotes, however the first 90 aminoacids are more variable and they are absent in *Arabidopsis thaliana* and *C.elegans*. The commercial antibody recognizes an epitope that has 66% of identity to the ortholog of CENP-V in *C.elegans*. Hence, two possible future experiments could be to tag the F25B4.8 with GFP to study the localization of CENP-V since F25B4.8 has been never characterised in the worm and/or to test the antibody used in the human oocyte.

The depletion of CENP-V by RNAi feeding showed chromosomes aberrations at diakinesis, i.e, fragments, fusions and. A plausible explanation is that CENP-V is necessary for the DSB repair and crossover formation. In some cells we have found twelve DAPI bodies and in most of them more than the expected six which it suggests the complete separation of the bivalents, as it happens in the spo-11 mutant. Spo-11 a topoisomerase II-like enzyme that catalyses the formation of meiosis-specific DNA double-strand breaks (DSBs) [24]. Assuming DAPI bodies as chromosomes, less than six DAPI bodies correspond to chromosomes fusions which could be due to sister reparation instead through the homologous chromosome as observed in the rad-51 mutant. Rad-51 is the eukaryotic homolog of the bacterial DNA strand exchange protein RecA and it catalyses DNA strand exchange between homologous sequences. Thus, Rad-51 promote the physical exchange of DNA molecules required for successful crossover recombination [25]. SYP proteins are essential for normal meiotic recombination although the absence of these proteins during DSBs formation do not affect its normal timing, but synapsis does not occur. Nevertheless, throughout pachytene, RAD-51 foci stay and only starts to disappear in diplotene nuclei. It seems to be that this situation is followed by reparation of meiotic DSB between sister chromatids. This idea, the reparation of meiotic DSB, is supported by the presence of 12 intact univalents at diakinesis in SYP mutants. A key target of this response is the core SC component SYP-1, which is phosphorylated in response to unrepaired meiotic DSBs. Checkpoint-dependent SYP-1 phosphorylation safeguards the germline against persistent meiotic DSBs by channelling repair to the sister chromatid [15, 21, 22, 26, 27]. It will be interesting in the future to analyse the role of CENP-V in the Syp1 mutants.

In summary, in this work we combine the study of CENP-V in two different systems, *C.elegans* and human oocytes. We proved that the pellucid zone removal is a crucial step to make possible the entrance of CENP-V antibodies. Moreover, some improvements must be done regarding the healthiness' of the cells and the home-made antibody still need to be tested. In the other hand, we show for the first time that CENP-V it is essential in diakinesis in

C.elegans. Thus, we open a great door of studies of this important protein in the worm to complement the future analysis in the human egg.

Methods

Sample collection and culture. CENP-V has been studied using discarded material from in vitro reproduction treatment. These discarded materials are immature oocytes in the germinal vesicle (GV) stage and, sometimes, we could find oocytes entering metaphase I stage. Oocyte retrieval is performed in the clinic and the oocytes are kept in ORIGIO Sequential Fert Medium (CooperSurgical) for 2 hours at 37ºC. Next, oocytes were decumulated to verify the maturation of them. The immatures ones in GV stage are prepared to be transported to the CABIMER after most of granulosa cells has been removed. *C.elegans* was maintained in NGM medium (Biomol). To performed the RNAi essay, L4 worms were plated on NGM supplemented with ampicillin, tetracycline and IPTG. Bacteria inoculum was prepared in LB supplemented with ampicillin. 48h later, young adults were collected and the gonad was extracted to perform immunofluorescence.

Immunofluorescence and image acquisition. oocytes were fixed in 1.6% PFA (Sigma) 30min at room temperature. Wash in PBT (PBS 1x (Sigma) + 0.1% Triton-X100 (Sigma)) and leave oocytes overnight at 4º in PBT. Oocytes were blocked in 3% BSA-PBT (ThermoFisher Scientific) 1h at room temperature. After the blocking, incubate in primary antibodies overnight at 4º. Primary antibodies (in 3% BSA-PBT) used were as follows: rabbit anti-CENP-V (HPA042616; Sigma) at 1:100, rat anti-α tubulin (MCA78G; Bio-Rad) at 1:1000, human anti-ACA (Antibodies Inc.) at 1:100, mouse anti-CENP-V monoclonal (clone 1D9; Sigma) at 1:50. Wash in 3% BSA-PBT. Secondary antibodies (in 3% BSA-PBT) were used as follows: mouse anti-rabbit FITC (1090-02; Southern Biotechnologie) at 1:400, anti-rat Alexa Fluor 594 (ThermoFisher Scientific) at 1:400, goat anti-human IgG H&L (A-21445; Invitrogen; Alexa Fluor 647) at 1:200, goat anti-mouse Ig (H+L)-FITC human (1010-02; Southern Biotechnologie) at 1:200. Oocytes were washed in 3% BSA-PBT 2 times x 10min on shaker; 2 times in PBT only and 2 times in PBS-1X + 0.01% BSA for 10min on shaker. Oocytes were mounted in Vectashield (H-1000; Vector Laboratories) DAPI (Sigma) (2 drops) for imaging. Fixed oocytes were imaged with Microscope Zeiss Apotome. In some oocytes the pellucid zone was removed using 2 drops of tyrode's solution (Thermo Scientific Chemicals) and then washed in ORIGIO Sequential Fert Medium plus mineral oil. Oocytes were fixed in 1.6% PFA 30 min at room temperature. Wash oocytes one time during 5 min in 3% BSA-PBT on shaker. Blocking in 3% BSA-PBT 1h at room temperature and then, oocytes were incubated in primary antibodies (in 3% BSA-PBT) for overnight at 4º. Oocytes were washed in 3% BSA-PBT 2 times x 5min on shaker at room temperature. Incubate the oocytes in secondary antibodies (in 3% BSA-PBT) for 2h at room temperature on shaker (without pellucid zone oocytes used to get sticky). Wash in 3% BSA-PBT x 2 times 10 min on shaker at room temperature. Oocytes were mounted in Vectashield DAPI (2 drops) for imaging. All steps, in both methods, were realised in a humid chamber to avoid evaporation. Immunofluorescence and DAPI staining of *C.elegans* was performed as previously described [28].

Image analysis and quantification. Fixed oocytes were imaged with Microscope Zeiss Apotome and 63x objective. Images analyses of the z-stacks and maximal projections were performed in FIJI software [29]. Image acquisition was made by 200 nm for each z plane.

Statistics and reproducibility. The data processing, statistical analysis, plotting was performed in RStudio, Excel (© 2015 Microsoft 360) and PowerPoint. Data were expressed as mean ± SD and statistical differences were tested by a two-way Anova test (***$p < 0.001$, **$p<0.01$, *$p < 0.05$). Anova test between control and CENP-V was significant.

References

1. Tadeu, A.M., et al., *CENP-V is required for centromere organization, chromosome alignment and cytokinesis*. EMBO J, 2008. **27**(19): p. 2510-22.
2. Honda, Z., T. Suzuki, and H. Honda, *Identification of CENP-V as a novel microtubule-associating molecule that activates Src family kinases through SH3 domain interaction*. Genes Cells, 2009. **14**(12): p. 1383-94.
3. Nabi, D., et al., *CENP-V is required for proper chromosome segregation through interaction with spindle microtubules in mouse oocytes*. Nat Commun, 2021. **12**(1): p. 6547.
4. *Encuesta de fecundidad*. Instituto nacional de estadística, 2019.
5. *Demografía de Europa*. Instituto nacional de estadística, 2021.
6. Camaioni, A., et al., *The process of ovarian aging: it is not just about oocytes and granulosa cells*. J Assist Reprod Genet, 2022. **39**(4): p. 783-792.
7. Hunter, N., *Oocyte Quality Control: Causes, Mechanisms, and Consequences*. Cold Spring Harb Symp Quant Biol, 2017. **82**: p. 235-247.
8. Charalambous, C., A. Webster, and M. Schuh, *Aneuploidy in mammalian oocytes and the impact of maternal ageing*. Nat Rev Mol Cell Biol, 2023. **24**(1): p. 27-44.
9. Tang, W.W., et al., *Specification and epigenetic programming of the human germ line*. Nat Rev Genet, 2016. **17**(10): p. 585-600.
10. Haering, C.H., et al., *The cohesin ring concatenates sister DNA molecules*. Nature, 2008. **454**(7202): p. 297-301.
11. Kitajima, T.S., M. Ohsugi, and J. Ellenberg, *Complete kinetochore tracking reveals error-prone homologous chromosome biorientation in mammalian oocytes*. Cell, 2011. **146**(4): p. 568-81.
12. Verlhac, M.H. and M.E. Terret, *Oocyte Maturation and Development*. F1000Res, 2016. **5**.
13. Hassold, T., H. Hall, and P. Hunt, *The origin of human aneuploidy: where we have been, where we are going*. Hum Mol Genet, 2007. **16 Spec No. 2**: p. R203-8.
14. Hassold, T. and P. Hunt, *To err (meiotically) is human: the genesis of human aneuploidy*. Nat Rev Genet, 2001. **2**(4): p. 280-91.
15. Garcia-Muse, T. and S.J. Boulton, *Meiotic recombination in Caenorhabditis elegans*. Chromosome Res, 2007. **15**(5): p. 607-21.
16. Pazdernik, N. and T. Schedl, *Introduction to germ cell development in Caenorhabditis elegans*. Adv Exp Med Biol, 2013. **757**: p. 1-16.
17. Villeneuve, A.M., *A cis-acting locus that promotes crossing over between X chromosomes in Caenorhabditis elegans*. Genetics, 1994. **136**(3): p. 887-902.

18. Goenrich, M., et al., *A glutathione-dependent formaldehyde-activating enzyme (Gfa) from Paracoccus denitrificans detected and purified via two-dimensional proton exchange NMR spectroscopy*. J Biol Chem, 2002. **277**(5): p. 3069-72.
19. Gupta, S.K., *The Human Egg's Zona Pellucida*. Curr Top Dev Biol, 2018. **130**: p. 379-411.
20. Kamath, R.S. and J. Ahringer, *Genome-wide RNAi screening in Caenorhabditis elegans*. Methods, 2003. **30**(4): p. 313-21.
21. Martin, J.S., et al., *RAD-51-dependent and -independent roles of a Caenorhabditis elegans BRCA2-related protein during DNA double-strand break repair*. Mol Cell Biol, 2005. **25**(8): p. 3127-39.
22. MacQueen, A.J., et al., *Synapsis-dependent and -independent mechanisms stabilize homolog pairing during meiotic prophase in C. elegans*. Genes Dev, 2002. **16**(18): p. 2428-42.
23. Maezawa, S., et al., *SCML2 promotes heterochromatin organization in late spermatogenesis*. J Cell Sci, 2018. **131**(17).
24. Dernburg, A.F., et al., *Meiotic recombination in C. elegans initiates by a conserved mechanism and is dispensable for homologous chromosome synapsis*. Cell, 1998. **94**(3): p. 387-98.
25. Alpi, A., et al., *Genetic and cytological characterization of the recombination protein RAD-51 in Caenorhabditis elegans*. Chromosoma, 2003. **112**(1): p. 6-16.
26. Colaiacovo, M.P., et al., *Synaptonemal complex assembly in C. elegans is dispensable for loading strand-exchange proteins but critical for proper completion of recombination*. Dev Cell, 2003. **5**(3): p. 463-74.
27. Garcia-Muse, T., et al., *A Meiotic Checkpoint Alters Repair Partner Bias to Permit Inter-sister Repair of Persistent DSBs*. Cell Rep, 2019. **26**(3): p. 775-787 e5.
28. Girard, L.R., et al., *WormBook: the online review of Caenorhabditis elegans biology*. Nucleic Acids Res, 2007. **35**(Database issue): p. D472-5.
29. Schindelin, J., et al., *Fiji: an open-source platform for biological-image analysis*. Nat Methods, 2012. **9**(7): p. 676-82.