

Characterisation of an oocyte specific knockout model of Cdh1

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Abbreviations

Approx.	approximately
BSA	bovine serum albumin
cAMP	cyclic adenosine 3'-5 monophosphate
CCD	charge coupled device
CEO	cumulus enclosed complex
Cntl	control
dbcAMP	dibutyl cAMP
dNTPs	Deoxyribonucleotide triphosphate
EDTA	Disodium Salt Dihydrate or Ethylenediaminetetraacetic acid
EGTA	Ethylene glycol-bis(2-Amino ethyl ether)-N,N, N',N'-tetra acetic acid
EtBr	Ethidium bromide
F	female
FCS	foetal calf serum
fl	floxed
FSH	follicle stimulating hormone
GV	germinal vesicle
GVB	germinal vesicle breakdown
H&E	Hematoxylin & Eosin
h	hour
hCG	human chorionic gonadotrophin
HEPES	N-(2-Hydroxyethyl) piperazine-N'-(2-ethanesulfonic acid)
Het	heterozygote
IVM	<i>in vitro</i> maturation
KO	knockout
LH	luteinizing hormone
MEM	minimal essential medium

mg	milligrams
MgCl	magnesium chloride
MI	meiosis I
MII	meiosis II
Mil	milrinone
Milli-Q	Distilled water filtered through 0.22 um Millipak®Express Millipore
Min	minutes
PBE	polar body extrusion
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PDE	phosphodiesterase
PFA	paraformaldehyde
Pgcs	primordial germ cells
PIPES	piperazine-N,N'-bis(ethanesulfonic acid)
PKA	protein kinase A
PMSG	pregnant mare's serum gonadotrophin
PVP	polyvinylpyrrolidone
Rpm	revs per minute
Rt	room temperature
SDS	sodium dodecyl sulfate
sec	seconds
TAE	Tris-Acetate-EDTA
µm	micrometer
µM	micromolar
V	volts
Wt	wildtype
Zona	zona pellucida

Abstract

Cdh1, a co-activator of the Anaphase Promoting Complex (APC) has recently been shown to be important in germinal vesicle stage arrest and in the prevention of aneuploidy during the first meiotic division of mouse oocytes. However, this was through antisense knockdown approaches done *in-vitro*. Therefore, here I generated an oocyte-specific knockout of Cdh1 (Δ/Δ) to explore this further. In this way, Cdh1 protein was specifically deleted only in germinal vesicle (GV) stage oocytes from growing follicles. Fewer cumulus enclosed oocytes were observed from Δ/Δ mice, of which significant numbers had undergone GVB. Furthermore, significantly more meiotically advanced, fragmented or degenerate oocytes were observed in knockouts. Denuded Δ/Δ GV oocytes also displayed a propensity for spontaneous GV breakdown (GVB) which could be partially rescued by maintaining an intact cumulus mass. Δ/Δ oocytes also underwent accelerated GVB on release from arresting media. Western Blots revealed a 5 fold increase in cyclin B1 levels following loss of Cdh1, whereas other substrates of APC^{Cdh1}, securin and Cdc25B, remained unchanged.

In-vivo and *in-vitro* matured metaphase II (MII) oocytes were analysed for aneuploidy rates. *In-vivo* matured knockout oocytes had higher, but not statistically significant, rates of aneuploidy than controls. Denuded oocytes that underwent IVM also had a

higher incidence of aneuploidy in knockouts and in this group this was highly significant. In summary, data from the *in-vivo* knockout model supports those of the *in-vitro* antisense approach and provide further evidence for the role of Cdh1 in both GV arrest and the prevention of aneuploidy, at least in the situation where oocytes are cultured *in-vitro*.

