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Full Length Article

The effect of cadmium on the bovine *in vitro* oocyte maturation and early embryo development

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ABSTRACT

Common pollutants such as heavy metals and cadmium is among those with high environmental concerns. In vivo studies had shown that cadmium (Cd) causes oocyte degeneration and embryo mortality, and lowers pregnancy rates in mammals. However, there is limited information available about direct effects of Cd on oocyte maturation and/or embryo development. This study was aimed to investigate if Cd has any effect on the oocyte maturation and/or embryo development *in vitro*. Bovine COCs were collected from the slaughter house and cultured for 24 h in serum-free media only (Controls) or supplemented with 0.2, 2.0 and 20.0 μM $CdCl_2$. At 24 h cumulus cell expansion was assessed in all COCs. COCs were either denuded and stained for determination of nuclear maturation or fertilized for assessment of subsequent embryo development. Cd at the lowest concentration (0.2 μM) did not affect any of the parameters studied. However, at higher concentrations (2.0 and 20.0 μM) it significantly ($P < 0.05$) reduced the percentage of fully-expanded COCs and significantly ($P < 0.05$) increased the percentage of partially and/or non-expanded COCs compared to controls and 0.2 μM. Cadmium at higher concentrations (2.0 and 20.0 μM) also significantly ($P < 0.01$) reduced the percentage of oocytes reaching metaphase II stage compared to controls and 0.2 μM. Post-fertilization cleavage rate in presumptive zygotes and blastocyst development significantly ($P < 0.05$) reduced 0.2, 2.0 and 20.0 μM $CdCl_2$ compared to the controls (0.0 μM). In conclusion, these results suggest that Cd had direct detrimental effects on the bovine oocyte maturation and its developmental competence.

1. Introduction

The possible exposure of humans and animals to many industrial chemicals and pesticides has been a growing concern over the last decade for both the scientific community and the general public. Several studies have suggested that these environmental contaminants could adversely affect reproductive functions and embryo abnormalities in animal populations [1–6]. Cadmium (Cd; atomic number 48; relative atomic mass 112.40) is one of the most toxic environmental and industrial heavy metals because of its long half-life (15–30 years) and its widespread occurrence. Relevant industrial Cd-emitting processes include the combustion of fossil fuels, leachate from landfill sites, run-off agricultural land, mining and smelting operations. Electroplating and manufacture of pigments, plastics, plastic stabilizers and nickel-Cd batteries also produce Cd as a by-product [6]. Moreover, it is also produced by the gradual process of erosion and abrasion of rocks and soils which are caused by events such as forest fires and volcanic eruptions [7,8].

The real problem with Cd is that it is not eliminated from ecosystems, mainly because of very long half-life but enters the food chain through environmental contamination of soil and is bioaccumulated [9]. Cadmium is excreted at a low rate from the body and is accumulated over time in various body organs including reproductive organs. Various sources of Cd exposure are reported, such as occupational, ecosystem-linked, diet-associated and non-occupational sources [10]. In cows and ewes, effects on various systems have been reported due to Cd present in feed and water [11,12]. Industrial cadmium (pesticides, batteries, tobacco, rubber processing) is strongly implicated in human and animal poisoning and its low-level exposure is a strong reproductive toxicant to humans too. Physiological concentrations of Cd in blood from cattle reared around different industrial/urban areas have been reported to range from 0.03 to 0.12 μg/mL [13], whereas in humans these values are 2.9 ± 2.5 mg/mL (human blood) [14], and 6.73 ± 0.31 ng/mL (follicular fluid) [15]. In several species, long-term

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exposure to Cd causes organ damage or functional deficiency [16] and in female mammals Cd affects ovarian function both directly and indirectly [17–19].

Oocyte maturation is crucial for nuclear maturation, successful fertilization and embryo development [20,21]. Oocyte maturation is a process during which the oocyte acquires its intrinsic ability to support the subsequent stages of development in a stepwise manner, ultimately reaching to the activation of embryonic genome. This process involves complex and distinct, although linked, events of nuclear and cytoplasmic maturation [22]. Nuclear maturation involves resumption of meiosis, progression to metaphase II (MII), and the accompanying cytoplasmic changes that prepare the oocyte for fertilization and subsequent embryonic development [23].

Cadmium being an environmental contaminant has been reported to be toxic, carcinogenic [24,25] and mutagenic [25]. Cadmium chloride has been shown to be toxic to the cumulus oocyte complexes [26]. Oocyte development and associated events have been disrupted by Cd administration in different species [6]. The role of Cd in suppressing FSH-induced cumulus expansion in oocyte–cumulus complexes (OCC) isolated from large antral porcine follicles has been described by Mlynarcikova et al. [21]. High concentrations of Cd completely suppressed oocyte maturation and also significantly suppressed an integral component of expanded cumulus cells in porcine oocytes [27].

There is limited number of studies done on the effect of Cd on the processes of maturation and fertilization of the oocyte. Therefore, the aim of this study was, to investigate, *in vitro* effect of Cd on the bovine oocyte maturation, (IVM) fertilization (IVF), and subsequent embryo development.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were purchased from Sigma Chemical Company unless otherwise stated.

2.2. Experiments

Water soluble Cadmium chloride ($CdCl_2$) was used as a source of Cadmium (Cd). Four different concentrations 0.0 μM, 0.2 μM, 2.0 μM and 20 μM $CdCl_2$ were added to serum-free oocyte maturation medium (M-199). In this study, a total of 1081 Cumulus-oocyte complexes (COCs) (grade 1; with homogenous ooplasm and more than four complete layers of cumulus cells) were used. COCs (n: 331) were used for checking nuclear maturation and 360 COCs were used for fertilization and/or cleavage rate of presumptive zygotes.

In the 1st experiment, effect of increasing concentrations of 'Cd' on oocyte nuclear maturation was studied. A total of 331 COCs were used in three independent repeats, allocating about 25–30 oocytes per replicate (0 μM Control, 0.2 μM, 2.0 μM and 20 μM $CdCl_2$). Cumulus cell expansion was recorded after 24 h of culture in all 1081 oocytes, and assessment of nuclear maturation of oocytes was done by fixation and staining.

In the 2nd experiment, effect of increasing concentrations of 'Cd' in the oocyte maturation medium was studied on the cleavage rate and blastocyst formation. A total of 360 oocytes were used in three independent repeats, divided into four treatments having about 20–25 oocytes per replicate. Oocytes were matured in the serum-free medium supplemented with either 0 μM controls, or 0.2, or 2.0, or 20 μM $CdCl_2$. The matured oocytes were fertilized using frozen semen. Cleavage rate was recorded on day 2 of culture, and blastocyst formation was recorded on day 8 of culture.

2.3. Collection of ovaries and oocytes

Cow ovaries collected from a local slaughter house were transported to the laboratory, within 2 h after slaughtering, in a thermos bottle containing 1 × PBS at 37–38 °C. The ovaries were washed with fresh 1X PBS several times immediately after arrival in the laboratory. Cumulus-oocyte complexes (COCs) were aspirated from follicles of 3–8 mm in diameter with an 18-gauge needle using 10 mL syringe. The grade 1 COCs were selected under a stereomicroscope and washed two times in M-199 supplemented with 25 μM HEPES and 0.4% (w/v) BSA.

2.4. In vitro maturation

Oocytes were matured in four-well dishes (NUNC, Thermo Fisher Scientific, Loughborough, Leicestershire, UK) containing 20 μL serum-free maturation medium (M-199 supplemented with 0.6% (w/v) fatty acid-free BSA, 10 mg/mL FSH (Follitropin; Bioniche Animal Health, Belleville, Ontario, Canada), 10 mg/ml LH (Leutropin; Bioniche Animal Health), 10 mg/mL oestradiol and 10 mg/mL gentamycin) for 24 h at 38.5 °C under 5% CO_2 in humidified air [28].

2.5. Assessment of cumulus cell expansion

Expansion of cumulus cells was recorded after 24-h of maturation under a stereomicroscope as described previously [29]. Levels of expansion recorded were a). Fully expanded (all cumulus cells were loosened or spreaded), b). Partially expanded (only outer layers of cells were loosened or spreaded) and c). Not expanded.

2.6. Assessment of stage of nuclear maturation of oocytes by staining

Nuclear maturation of oocytes was assessed by staining of oocytes as previously described Marei et al. [29]. All oocytes were denuded and placed on a slide holding the cover slip supported by four droplets of vaseline/paraffin mixture (40:1). Fixation of denuded oocytes was done by placing the slides in acetic acid:methanol fixative (1:3 v/v) for at least 48 h. For staining, aceto-orcein stain (1% orcein in 45% acetic acid) was used. Nuclear maturation of the oocytes was determined according to the morphology of the nuclear material under phase contrast microscope (Leica, Milton Keynes, UK).

2.7. In vitro fertilization

Oocytes matured *in vitro* with maturation medium containing 0, 0.2, 2.0 and 20 μM $CdCl_2$ were fertilized with frozen semen (gift from Genus ABS, Nantwich, Cheshire, UK) from a single bull as previously described [18]. Sperms were selected by swim up for 45 min at 38.5 °C under 5% CO_2 in humidified air in Calcium-free medium. After that, supernatant was centrifuged at 300*g* at 20 °C and pellet was re-suspended in fertilization medium (Tyrode's albumin-lactate-pyruvate media supplemented with 0.6% (w/v) fatty acid-free BSA, 1 mg/mL heparin, 50 ng/mL adrenaline and 50 ng/mL hypotaurine). For removing or de-aggregating cumulus cells, COCs were gently pipetted leaving only few layers of cumulus cells. Nearly 30 of these COCs were washed with oocyte wash medium once and then transferred into 400 mL of fertilization medium containing 1 × 10^6 sperm/mL. These cultures were incubated for 18 h at 38.5 °C in a humidified incubator of 5% CO_2 in air.

2.8. Embryo culture

After gentle pipetting, presumptive zygotes were denuded from cumulus cells and cultured in 500 mL of synthetic oviductal fluid medium containing amino acids, sodium citrate and myoinositol [30] supplemented with 0.4% (w/v) fatty acid-free BSA at 38.5 °C in a humidified incubator with 5% O_2, 5% CO_2 and 90% N_2. This was considered as day 1 (fertilization as day 0) of culture and continued up to day 8 for the formation of blastocyst. Cleavage rate and blastocyst development was measured on day 2 and 8 of culture, respectively.