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Effect of glucocorticoids on spontaneous and follicle-stimulating hormone induced oocyte maturation in mouse oocytes during culture☆

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Abstract

Several studies have indicated that glucocorticoids are involved in maturation of mammalian oocytes. Recently, maturation of porcine oocytes in culture was shown to be inhibited by glucocorticoids in a time- and dose-dependent manner. In addition, levels of cortisol available for biological action in fluid of preovulatory follicles are higher than that present in circulation. The present study evaluates the effect of cortisol and dexamethasone on mouse cumulus enclosed oocytes (CEO) undergoing spontaneous- and FSH-induced maturation during a 24 h culture period using breakdown of the germinal vesicle (GVBD) as end-point. FSH-induced oocyte maturation was studied using media containing 4.5 mM hypoxanthine to maintain levels of cAMP elevated, whereas spontaneous oocyte maturation was studied in a medium without hypoxanthine. In the presence of FSH (25 IU/l) the rate of GVBD was significantly elevated compared to the control. Dexamethasone (1–20 μg/ml) in combination with FSH resulted in a rate of GVBD similar to FSH alone. Cortisol (0.1–10 μg/ml) resulted in a significant higher rate of GVBD in combination with a physiological concentration of FSH (10 IU/l) as compared to the control but similar to that caused by FSH alone. Nearly all CEO that matured spontaneously resumed meiosis irrespective of whether or not cortisol was present. In conclusion, these results indicate that glucocorticoids have little or no influence on the regulation of oocyte maturation in the mouse. Species differences between mouse and pig oocytes may exist.

Keywords: Oocyte maturation; Resumption of meiosis; Cortisol; Dexamethasone; Meiosis activating sterol (MAS)

1. Introduction

The meiotic division of follicle-enclosed oocytes is arrested in the prophase of the first meiotic division until shortly before ovulation, where oocytes of large preovulatory follicles acquire competence to resume meiosis in response to the mid-cycle surge of gonadotropins. At follicular rupture the oocytes has reached the metaphase of the second meiotic division and are capable of sustaining fertilization and further development. The meiotic status of mammalian oocytes can usually be assessed at the microscopic level, since the nuclear membrane, the so-called germinal vesicle (GV) is visible in oocytes that still rest in the prophase of the first meiotic division. The nuclear membrane disappears in oocytes that have initiated resumption of meiosis and such oocytes are said to have undergone germinal vesicle breakdown (GVBD). A large number of studies have used this fact to evaluate the mechanisms by which resumption of meiosis is regulated.

The central role of gonadotropins in the regulation of oocyte maturation is now well established, but signal transduction following gonatodotropin receptor activation is still poorly understood. It is generally believed that cAMP has an important role in the regulation of oocyte maturation, since high levels maintain meiotic arrest, whereas reduced levels coincide with resumption of meiosis. Removing the oocyte from the follicular compartment and the influence of high cAMP result in GVBD, i.e. spontaneous maturation (review [1,2]). Conversely, it has been shown that the follicle in an environment that maintain high levels of cAMP is capable of producing signals that positively overcome the meiotic arrest [3,4]. Thus, isolated cumulus oocyte complexes stimulated with FSH will overcome the meiotic arrest caused by high levels of cAMP. The nature of this FSH induced signal has not yet been identified, but recently it has been shown that a class of sterols promotes oocyte maturation similar to FSH and is likely to be part of the FSH induced signal transduction pathway leading to resumption of meiosis [5]. Initially

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two substances isolated from human follicular fluid (i.e. 4,4-dimethyl-5a-cholesta-8,14,24-triene-3β-ol) and bull testicular tissue (i.e. 4,4-dimethyl-5a-cholesta-8,24-diene-3β-ol) were shown to induce resumption of mouse oocytes in vitro and hence given the names FF- and T-MAS, respectively [5,6]. However, the precise mechanism by which FSH and MAS induce resumption of meiosis has not yet been determined.

Glucocorticoids diffuse from circulation to the follicle fluid surrounding the oocyte and intrafollicular levels of free biological active cortisol is usually higher than those in the blood [7,8]. Glucocorticoids have previously only indirectly been associated with oocyte maturation in humans [9–12], but recent studies in pigs have shown that dexamethasone and cortisol had a direct inhibitory effect on the rate of oocytes that underwent GVBD during culture [13], being the first mammalian oocytes evaluated in this context in more detail. In addition, further studies by the same group identified a potential mechanism within the oocyte responsible for the inhibitory actions of dexthametasone and cortisol [14], since it was demonstrated that these glucocorticoids decreased the levels of cyclin B1, which is an essential part of M-phase promoting factor (MPF). M-phase promoting factor acts as an important cell-cycle regulatory protein with kinase activity and is downstream to cAMP activation. Activation of MPF is also involved in resumption of meiosis in oocytes [15]. However, recently it has been shown that MAS induced oocyte maturation was only little sensitive to inhibition of MPF activity [16] and it is presently unknown whether FSH/MAS induced oocyte maturation is affected by glucocorticoids.

The aim of the present study was (1) to evaluate the effect of glucocorticoids (i.e. cortisol and dexamethasone) on maturation of mouse oocytes in culture. These studies included FSH-induced oocyte maturation attempting to mimic the in vivo situation by keeping levels of cAMP high in the presence of hypoxanthine (HX) and to study the effect of glucocorticoids on spontaneous maturation of oocytes (i.e. HX is not present in the culture medium) (2) to approach whether FSH, MAS and glucocorticoids interacted during regulation of oocyte maturation.

2. Material and methods

2.1. Animals and basic culture media

Immature female mice (C57Bl/2J or B6D2-F1, Bomholtgård, Denmark) were kept in a temperature-controlled room (20 ± 2 °C) with a 14–10 h light-dark cycle (lights on from 06.00 to 20.00 h) with free access to food and water.

Ovarian stimulation was performed when the mice weighed 10–16 g and consisted of one intraperitonal administration of 15 IU Menogon (Ferring, Copenhagen, Denmark) containing 15 IU FSH and 15 IU LH-like activity. The animals were sacrificed 46–48 h later by cervical dislocation and the ovaries removed to dishes with culture medium supplemented with 4.5 mM hypoxanthine (HX) (Gibco, BRL), termed HX-medium. The medium consisted of α-Minimal Essential Medium without nucleosides and deoxyribonucleosides, (α-MEM, Gibco, BRL), 3 mg/ml bovine serum albumin (BSA) (both from Sigma), 5 mg/ml human serum albumin (HSA) (Statens Seruminstitut, Copenhagen, Denmark), 2 mM L-glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin (all from Gibco, BRL). Ovaries were dissected free of surrounding tissues using 27-gauge needles and transferred to a small petri-dish with fresh HX-medium. The medium used to study spontaneous maturation was similar to the HX-medium, but did not contain HX. The medium was named spon-medium. The experiments were performed according to the rules of the Danish Authorities for Animal Care, Ministry of Justice.

2.2. Culture of oocytes (the oocyte assay)

Antral follicles were punctured under a dissecting microscope using 27-gauge needles. Cumulus enclosed oocytes (CEO) of equal size with a uniform layer of cumulus cells were collected. The oocytes were collected in fresh HX-medium and washed twice (i.e. either HX-medium or spon-medium depending on experiment) before transfer to the test medium. The oocytes were cultured at 37 °C, 100% humidity in 5.3% CO_2, 6.9% O_2 and 87.8% N_2 for 24 h in 4-well dishes (Nunclon, Roskilde, Denmark) in 500 μl culture medium. At the end of culture the oocytes were examined for their meiotic status using an inverted microscope with Hoffman modulation contrast equipment. Oocytes showing a clear nuclear membrane (germinal vesicle, GV) were classified as meiotically arrested whereas those showing no nuclear structures were classified as having undergone germinal vesicle breakdown (GVBD). The percent of GVBD (including PB) per total number of oocytes (% GVBD) and the percent of PB per GVBD (% PB) were calculated. Assays were repeated three times.

2.3. Test media

Dexamethasone and cortisol (both from Sigma, Vallensbæk, Denmark) were dissolved in ethanol and diluted appropriately. The final concentration in test media did not exceed 0.5% ethanol and ethanol in similar concentrations was added to control media. The FSH preparation used was from Organon (Puregon, Organon, Oss, The Netherlands).

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. and for statistical analysis STATGRAPHICSTM software (Manugistics, Inc., Rockville, MD, USA) was applied. The frequency of GVBD in test and control media was compared after appropriate transformation using the Student's *t*-test.

Table 1
Effect of dexamethasone on FSH induced oocyte maturation in mouse oocytes

	No. of experiments	No. of oocytes	GVBD (%) ± S.E.M.	PB/GVBD (%) ± S.E.M.
Control	6	166	49 ± 3	42 ± 5
FSH (25 IU/l)	5	157	77 ± 5	52 ± 10
FSH (25 IU/l) + 1 μg/ml dexamethasone	5	162	78 ± 4	47 ± 11
FSH (25 IU/l) + 5 μg/ml dexamethasone	5	156	77 ± 3	43 ± 7
FSH (25 IU/l) + 10 μg/ml dexamethasone	6	181	78 ± 5	35 ± 9
FSH (25 IU/l) + 20 μg/ml dexamethasone	2	59	72 ± 4	38 ± 4
Dexamethasone without FSH[a]	5	145	44 ± 3	35 ± 15

[a] Experiments with dexamethasone without FSH addition used concentrations of dexamethasone of 10 μg/ml ($N = 2$), 5 μg/ml ($N = 2$) and 1 μg/ml ($N = 1$) with similar results for the different concentrations.

3. Results

Using a total of 2216 mouse cumulus enclosed oocytes the effect of dexamethasone and cortisol on FSH-induced- and spontaneous oocyte maturation was evaluated. The effect of dexamethasone on FSH-induced oocyte maturation is shown in Table 1. The percentage of oocytes that underwent GVBD in the control group was 49% rising to 77% in the positive control that was stimulated with FSH (25 IU/l) ($P < 0.05$). Increasing the concentration of dexamethasone from 1 to 20 μg/ml had no effect on the rate of GVBD, which remained at the exact same level as that of the positive control. Dexamethasone itself showed no effect on the rate of GVBD being at the same level as the negative control. The frequency by which polar body extrusion occurred remained constant in the different groups.

The effect of cortisol on FSH-induced oocyte maturation is shown on Fig. 1. The rate of GVBD is essentially similar to that of the dexamethasone experiment, showing that the negative control resulted in a rate of GVBD of 41% rising to 78% in the positive control ($P < 0.05$), which was FSH at a concentration of 10 IU/l. Cortisol in concentrations of 0.1–10 μg/ml showed no effect on the FSH induced oocyte maturation and cortisol itself (10 μg/ml) was without effect on the rate of GVBD (i.e. similar to the negative control).

Using a total of 496 CEO the effect of cortisol on spontaneous oocyte maturation was evaluated (Fig. 2). During a 24 h culture period almost all oocytes underwent GVBD irrespective of whether or not cortisol was present.

4. Discussion

This study demonstrates that glucocorticoids (i.e. dexamethasone and cortisol) were without any effect on the frequency by which mouse CEO resume meiosis (FSH-induced and spontaneous maturation) during a 24 h culture period. The range of cortisol concentrations tested include physiological concentrations as well supraphysiological levels [7,8]. Results using a concentration of FSH of 25 IU/l in combination with dexamethasone or FSH (10 IU/l) in combination with cortisol were remarkably similar, and showed no sign of either inhibitory or stimulatory effect on the rate of GVBD. A concentration of FSH at this level (10–25 IU/l)

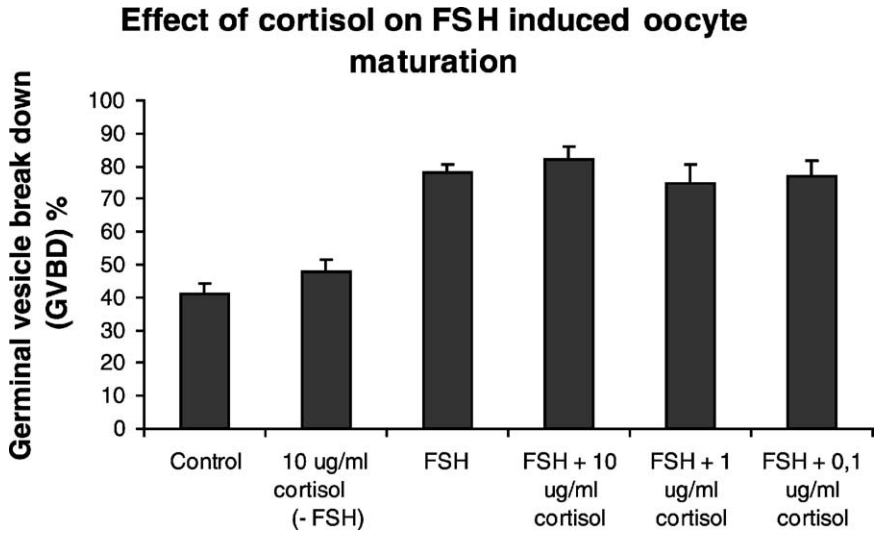

Fig. 1. Mouse cumulus enclosed oocytes cultured for a period of 24 h. FSH was used in a concentration of 10 IU/l. Results are given as mean ± S.E.M.

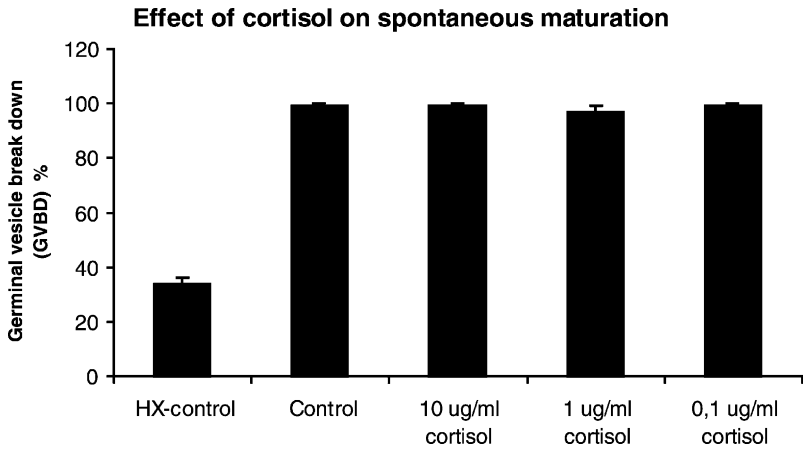

Fig. 2. Mouse cumulus enclosed oocytes were cultured for a period of 24 h. Results are given as mean ± S.E.M.

is only slightly higher than the physiologically observed levels and suggests that glucocorticoids have only little if any effect on the resumption of meiosis that occur in mouse oocytes in response to the mid-cycle surge of gonadotropins.

Receptors for glucocorticoids are located in the ovary [17] and a number of studies have shown that glucocorticoids affect steroidogenesis, resulting in both inhibitory and stimulatory effects [18]. Cortisol has been reported to reduce FSH-stimulated estrogen biosynthesis and reduce pregnenolone synthesis by inhibition of the cholesterol side-chain cleavage enzyme [19,20], whereas others have reported enhanced progesterone production by rat granulosa cells, probably by stimulation of 3β-hydroxysteroid dehydrogenase (3βHSD) and inhibition of 20αHSD [21]. Other studies have shown that glucocorticoids in rat and human granulosa cells that have not luteinized in vitro seem to potentate the steroidogenic responses by LH and FSH probably acting through the steroidogenic enzymes and by enhancing cAMP signal transduction [18]. Taken together, there is strong evidence to suggest that glucocorticoids affect ovarian steroidogenesis by altering activities of the enzymes participating in biosynthesis. However, the specific effect of glucocorticoids may be related to the stage of menstrual cycle and the differentiation state of the cell [18].

The discovery of MAS, which overcomes the meiosis inhibitory effect of hypoxanthine and induce oocytes to resume meiosis, identified a sterol/steroid that directly affected the meiotic division and for the first time linked steroidogenesis and oocyte maturation [5]. It has been suggested that a local accumulation of MAS is part of the physiological signal transduction pathway that leads to oocyte maturation [22]. It is, however, unknown how MAS may be accumulated and several mechanisms have been proposed [22,23]. One attractive hypothesis was suggested by Lindenthal et al. [23], who showed that intrafollicular concentrations of progesterone and 17α-hydroxyprogesterone caused MAS accumulation in cells of the rat testis [23]. It was proposed that ovarian steroids caused a negative feed-back inhibition of steroidogenic enzymes leading to MAS accumulation, reflecting events taking place during the mid-cycle surge of gonadotropins, where progesterone and 17α-hydroxyprogesterone accumulates. This particular hypothesis by Lindenthal et al. [23] did, however, not find support by actual studies on oocyte maturation in vitro [24], since progesterone and 17α-hydroxyprogesterone was without effect on oocyte maturation. Nevertheless, the concept of steroids affecting enzymes earlier in steroidogenic pathway remain attractive and in light of the reported effects of glucocorticoids on steroidogenesis in combination with the hefty increase in cortisol available for biological action in connection with ovulation [7,8], this could potentially represent a physiological mechanism whereby glucocorticoids could interact with the regulation of oocyte maturation. Therefore, an effect of glucocorticoids on the rate at which oocytes resumed meiosis, either as a result of FSH stimulation or during spontaneous maturation, could possibly be mediated through an altered MAS accumulation. However, in both cases glucocorticoids were without effect on the rate at which oocytes resume meiosis and glucocorticoids only seem to have little if any effect on the regulation of meiosis in mouse oocytes.

Furthermore, the inhibitory effect of glucocorticoids on the expression of cyclin B and thereby the activity of the MPF and hence the rate of GVBD as reported for porcine oocytes [13] does not seem to apply to mouse CEO.

This study also shows that cortisol is without effect on the spontaneous maturation of mouse CEO during a 24 h culture period. The present study is therefore unable to support previous studies on pig oocytes, which showed a dose dependant inhibition of the rate of GVBD by cortisol and dexamethasone [13,14]. The differences between mouse and pig oocytes in their response to glucocorticoids are not readily explained. However, the experimental setup was different between the two studies [13]. The medium used in the pig oocyte studies contained high concentrations of LH, FSH and oestradiol. In addition, the medium did not contain

hypoxanthine. Furthermore, the culture medium was covered with a layer of mineral oil, which has a tendency to absorb the highly lipophilic steroids. Although the overall regulation of mammalian oocyte maturation is likely to similar in mammalian species more subtle changes may exist between species. Actually, there are data to suggest that porcine cumulus cells bind and respond to LH stimulation [25], whereas mouse cumulus cells are unresponsive to LH stimulation and LH do not affect maturation of mouse CEO [4,26]. Therefore, species differences seem to exist with regard to LH receptor expression and that may be reflected in the responsiveness of oocyte maturation to glucocorticoids.

In conclusion, the present study demonstrates that glucocorticoids are unable to affect the rate at which mouse CEO resume meiosis, either in response to FSH stimulation or to spontaneous maturation, and it is likely that glucocorticoids play no major role in connection with oocyte maturation in the mouse.

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