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Steroid hormones in bovine oviductal fluid during the estrous cycle

Julie Lamy [a], Philippe Liere [b], Antoine Pianos [b], Fanny Aprahamian [b], Pascal Mermillod [a], Marie Saint-Dizier [a,c,*]

[a] PRC, INRA, CNRS, IFCE, Université de Tours, Nouzilly, France
[b] UMR 1195 INSERM, Petites molécules de neuroprotection, neurorégénération et remyélinisation, Université Paris-Saclay, Université Paris-Sud, Le Kremlin-Bicêtre, France
[c] UFR Sciences et Techniques, Université François Rabelais de Tours, Tours, France

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ABSTRACT

Ovarian steroid hormones are major regulators of the physiology of the oviduct and reproductive events occurring within the oviduct. To establish a whole steroid profiling of the bovine oviductal fluid (OF) during the estrous cycle, contralateral and ipsilateral (to the corpus luteum or preovulatory follicle) oviducts were classified into four stages of the estrous cycle (n = 18–27 cows per stage): postovulatory (Post-ov), mid-luteal (Mid-lut), late luteal (Late-lut), and preovulatory on the basis of the ovarian morphology and intrafollicular steroid concentrations. Steroids were extracted from pools of 150 to 200 μL OF (three to 10 cows per pool; three to four pools per "stage × side" group), purified, fractioned by high-performance liquid chromatography, and analyzed by gas chromatography coupled with tandem mass spectrometry. The concentrations of progesterone (P4) in ipsilateral OF increased from Post-ov (56.9 ± 13.4 ng/mL) to Mid-lut (120.3 ± 34.3 ng/mL), then decreased from Late-lut (76.7 ± 1.8 ng/mL) to Pre-ov (6.3 ± 1.7 ng/mL), and were four to 16 times higher than in contralateral OF. Most P4 metabolites followed similar patterns of variation. Concentrations of 17beta-estradiol (E2) were significantly higher at Pre-ov (290.5 ± 63.2 pg/mL) compared with all other stages (<118.3 pg/mL), with no difference regarding the side of ovulation. Concentrations of androstenedione displayed a pattern similar to that of E2, whereas other androgens, estrone, and corticoids did not vary between stages or sides. In conclusion, a highly concentrated and fluctuating hormonal environment was evidenced in the bovine OF. These results could be useful to improve media for IVF, embryo development, and culture of oviductal cells.

1. Introduction

Important reproductive events take place in the mammalian oviduct, including the final maturation and transport of gametes, fertilization, early development, and transport of embryos toward the uterus [1–3]. The bovine oviduct undergoes physiological changes that contribute to establish an optimal environment for gametes and embryos during the estrous cycle [4,5]. The ovarian steroids 17beta-estradiol (E2) and progesterone (P4) are major regulators of these changes in the oviduct. Estrogens stimulate oviductal contractions, oviductal secretions, and increase the proportion of ciliated cells in the oviductal epithelium, whereas P4 is associated with a reduction of ciliated cells and decreased secretory activity and motility of the oviduct [6–8]. Numerous ions, amino acids, and energy substrates are produced in the tubal fluid, which can be modulated by P4 in the cow [9–11]. P4 was also reported to be a chemoattractant for spermatozoa in rabbit

* Corresponding author. Tel.: +33 247367014; fax: +33 247367094.
E-mail address: marie.saint-dizier@univ-tours.fr (M. Saint-Dizier).

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[12] and human [13]. Numerous steroid receptors are known to be expressed in the bovine oviductal tissue. The nuclear estrogen receptor (ER) α is present in bovine oviduct epithelial cells (BOECs) *in vivo* [14], and ERα and ERβ are present *in vitro* [15]. Nuclear and membrane P4 receptors are also expressed in bovine epithelial cells *in vivo* [14,16] and *in vitro* [15]. Androgen receptors were not reported in the bovine oviduct, yet they were described in human [17], mouse [18], and rat [19] oviducts. Furthermore, the gene expression in the BOECs was shown to change according to the stage of the estrous cycle [20] and the side relative to the corpus luteum in the postovulation period [21]. *In vitro*, addition of E2 and P4 in the culture medium was associated with transcriptomic changes in BOECs [15], and the addition of various concentrations of these steroids in the IVF medium was reported to increase the yield and quality of embryos obtained in the bovine species [22]. However, the concentrations of steroids to which bovine oviduct epithelial cells, gametes, and embryos are exposed *in vivo*, i.e., their topical concentrations in the bovine oviductal fluid (OF), are currently unknown.

Local countercurrent transfer of ovarian steroids from the ovary to the ipsilateral oviduct was reported in the cow, ewe, sow, and woman [23]. Concentrations of P4 and E2 as measured by immunoassay in the bovine oviductal tissue were reported to vary according to the stage of the estrous cycle and the side relative to the corpus luteum [24]. In the latter study, the highest concentrations of P4 (~500 ng/g of tissue) were measured in the oviducts ipsilateral to the corpus luteum during the luteal phase, whereas levels of E2 were highest (~900 pg/g) in the oviducts ipsilateral to the preovulatory (Pre-ov) follicle before ovulation. However, although numerous steroids, including P4, E2 [25,26], estrone [27], androgens [25,28], and cortisol [29], vary in plasma across the bovine estrous cycle, no information is currently available on their concentrations in the bovine OF. Nevertheless, a study reported that OF collected just before or after ovulation displayed significantly different concentrations of P4 and E2 in sows [30]. Moreover, concentrations of P4 recently measured by radioimmunoassay in one pool of equine OF were higher in the ipsilateral than in the contralateral side relative to ovulation, whereas the three other steroids measured (E2, estrone, and testosterone) were at similar concentrations irrespective of the side of ovulation [31].

In the present study, we used gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) to determine concentrations of all detectable steroids in the bovine OF collected at four different stages of the estrous cycle. GC-MS/MS is today the most powerful technology in terms of selectivity and sensitivity to establish a whole steroid profiling, i.e., progestogens, androgens, estrogens, corticoids, and their precursors and metabolites in a biologic tissue or fluid [32].

2. Materials and methods

2.1. Collection and preparation of samples

2.1.1. Collection of bovine OFs

Both oviducts and ovaries from individual adult cows were collected in a local slaughterhouse (Vendôme, France), immediately placed on ice and transported to the laboratory. The oviducts were classified into one of four stages of the estrous cycle on the basis of the morphology of ovaries and corpus luteum, as previously described [33]: postovulatory (Post-ov, days 1–5), early-to-mid luteal phase (Mid-lut, days 6–12), late luteal phase (Late-lut, days 13–18), and Pre-ov phase (days 19–21). The oviducts were also separated into ipsilateral (to Pre-ov follicle, ovulation site, or corpus luteum) and contralateral sides. Two animals with bilateral ovulations were included (one in the Pre-ov group and one in the Post-ov group), which provided each two ipsilateral samples. In addition, in the Pre-ov group, the follicular content of the Pre-ov follicle was aspirated with a syringe and the volume determined, and then 1-mL sample of follicular fluid was stored at −80 °C until steroid measurement. The oviducts were cleaned of surrounding tissues, and the content was collected by gentle squeezing (applying pressure) with a glass slide. Oviductal cells were then separated from the OF by centrifugation at 2000 × *g* for 5 minutes at 4 °C. The supernatants were then centrifuged for 5 minutes at 6000 × *g* at 4 °C. The remaining supernatants (20–100 μL per oviduct) were stored at −80 °C.

2.1.2. Exclusion of animals with ovarian cysts or atretic follicles in the Pre-ov group

To exclude animals with ovarian cysts from the Pre-ov group, all oviducts attached to an ovary with a follicle larger than 20 mm in diameter were discarded at the time of oviduct collection. To exclude remaining animals with ovarian cysts or atretic follicles, concentrations of P4 and E2 were measured in duplicate in Pre-ov follicular fluids (n = 31). Concentrations of P4 were measured by a competitive enzyme-linked immunosorbent assay according to a method previously described in Rico et al. [34], whereas E2 concentrations were measured using the BioSource E2-EASIA kit (BioSource Europe S.A., Louvain-la-Neuve, Belgium) as previously described in Adib et al. [35].

On the basis of the previous data obtained in the cow [36–38], animals with intrafollicular concentrations of P4 more than 160 ng/mL, of E2 lower than 40 ng/mL, and/or with a ratio of E2:P4 concentrations less than one were excluded from the Pre-ov group. Finally, the mean intrafollicular concentrations of P4 and E2 in the Pre-ov group were 58.8 ± 9.6 ng/mL (12.0–160.0 ng/mL) and 1302.3 ± 212.0 ng/mL (76.0-3173.7 ng/mL, n = 22), respectively (see Table 1 for details).

2.1.3. Pooling of OFs

Preliminary experiments reported that 150 μL was the minimal volume required for the detection by GC-MS/MS of estrogens in bovine OF at all stages. To reach at least three samples per estrous cycle stage and side, and on the basis of a mean volume of 30 μL per oviduct, a minimum of 18 animals were collected per condition before the day of sample pooling. On this day, all OFs were thawed on ice and pools of three to 10 individual fluids were made to reach a final volume of 150 to 250 μL per sample (see Table 2 for details). So the various pools at a given stage contained samples from different animals. In the following, the term "sample" will refer to a pool of OFs. The samples were stored at −80 °C until further analysis.