Theriogenology xxx (2016) 1–8

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Review

The functional role of insulin in fertility and embryonic development—What can we learn from the bovine model?

D. Laskowski [a,*], Y. Sjunnesson [a], P. Humblot [a], G. Andersson [b], H. Gustafsson [a], R. Båge [a]

[a] Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b] Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

Keywords:
Oocyte maturation
Gene expression
Metabolism
Blastocyst
Metabolic programming

A B S T R A C T

Insulin is a key metabolic hormone that plays a crucial role in regulating energy homeostasis in the body. In addition, insulin-dependent signaling has important functions in reproduction and early embryo development. As metabolism and reproduction are closely linked, metabolic challenges may be the source of reproductive disorders and decreased fertility. This is known for the dairy cow and for other species including the human. Although metabolic disorders in the dairy cow often derive from a failure to adapt to a high milk production, the situation in the human is often linked to emerging conditions and associated diseases in our modern society such as obesity and diabetes, where an excess energy intake causes decreased fertility in women. Both energy excess and energy deficit are associated with a deviation of insulin concentrations in serum and follicular fluid from normal levels. Although many studies have shown that extreme variation in energy supply can negatively influence early embryo development by inducing changes in circulating concentrations of several metabolites or hormones like insulin, several *in vitro* culture media are still supplemented with insulin in high concentrations. In this review, direct and indirect effects of insulin on fertility will be described. Differences between the *in vivo* and *in vitro* situations will also be discussed.

1. Introduction

Follicular growth including final maturation of the oocyte takes 8 to 12 weeks in cattle [1]. Several metabolites and hormones like insulin that exhibit concentration changes in serum according to the metabolic condition also change following the same pattern in the follicular fluid, although the concentrations may differ [2,3]. This implies that the oocyte may be exposed to changing metabolic conditions during development and its final maturation. All high-producing dairy cows experience more or less negative energy balance (NEB) in early lactation. The first insemination after parturition often occurs around 60 days postpartum. Thus, the oocyte presumed for fertilization starts to grow during the dry period before parturition. Most of its growth occurs while the cow is under an NEB environment, and most of the time, final maturation and ovulation occur when positive energy balance is resumed [4,5].

During periods of metabolic challenges such as NEB or overfeeding, insulin concentrations deviate from normal concentrations (around 0.2–0.6 ng/mL depending on study and measuring method, see also Table 1) and circulating levels are either decreased or elevated [6–8]. Insulin acts as a crucial metabolic signal in coupling the growth hormone, insulin-like growth factor (IGF) axis [9], which stimulates cell growth and proliferation. As the existence of insulin receptors has been confirmed in oocytes, cumulus cells [10], and in embryos from the zygote to the blastocyst stage [11], an influence of insulin on all these cell types and

* Corresponding author. Tel. +46-18-671870; fax: +46-18-671202.
E-mail address: denise.laskowski@slu.se (D. Laskowski).

0093-691X/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2016.04.062

Table 1
Insulin concentrations measured in follicular fluid and serum—a summary of the literature.

Insulin in plasma (ng/mL)	Insulin in follicular fluid (FF) (ng/mL)	Method/KIT	Reference	Additional information
0.21–0.34	—	ELISA, validated for cattle (Immuno-Biological Laboratories, Hamburg, Germany)	Vanholder et al. 2005 [64]	Lower value cyst, higher value normal ovulation
0.21–0.48	—	A specific double antibody RIA validated and described by reference in the article	Gong et al. 2002 [21]	
0.24 (heifer), 0.51 (cow)	—	Insulin auto DELFIA (solid-phase fluroimmunoassay, Tuku, Finland)	Bender et al. 2010 [65]	
0.32–0.40	—	125I-labeled insulin, double-antibody RIA based on a reference method in the article	Garnsworthy et al. 2009 [32]	
0.349–0.712 (luteal phase = 0.349; follicular phase = 0.417; peak = 0.712; mean = 0.5–0.69)	0.127–0.282 subordinate/ preovulatory	Coat-A-Count kit (DPC, Los Angeles, CA, USA), designed for human insulin analyses and validated for cattle by [66]	Landau et al. 2000 [3]	FF/preovulatory follicle: 0.3–0.72, FF/subordinate follicle: 0.12–0.17
0.4–0.6	—	EIA	Kawashima et al. 2007 [67]	No difference between ovulatory and anovulatory cows in this study
0.42–1.88	—	125I-labeled insulin double-antibody RIA based on a reference method in the article	Adamiak et al. 2005 [31]	Hyperinsulinemic (if >37.2 μIU/mL = 1.44 ng/mL) result in fewer oocytes/ blastocysts
—	0.38–0.42	By EIA, not specified	Shimizu et al. 2008 [36]	
—	0.5–10	—	Spicer and Echternkamp 1995 [2]	Content in FF lower or equal to plasma
—	0.27 (cyst), 1.15 (normal)	RIA (RIAK-1 kit, BRIT, Navi Mumbai, India)	Khan et al. 2011 [68]	Water buffalo
—	0.59 (acyclic), 1.01 (cyclic)	RIA (RIAK-1 kit, BRIT, Navi Mumbai, India)	Khan et al. 2012 [69]	Water buffalo

Abbreviation: EIA, enzyme immunoassay.

embryos can be expected. Extreme insulin levels could be detrimental for the developmental potential of the oocyte and the cause of poor pregnancy outcome. This leads not only to economic losses for the farmer but may also impair the health status of live offspring later in life due to an unfavorable environment during early embryonic development. Interestingly, children born during a period of food deprivation [12] suffer later in life from the same diseases and metabolic disorders as children from obese mothers [13]. Part of the numerous health defects reported may be due to the dysregulation of energy metabolism during oocyte maturation and early development. We can only speculate about similarities between long time effects shown with obese and diabetic nonbovine mothers, as it has still not been proven in cattle that NEB negatively affects offspring health. The metabolic programming induced by epigenetic changes due to an environment with energy excess or deprivation during the periconception period can affect the offspring's health and body condition during their whole life [14]. Taking into account the strong environmental impact of reproductive tissues and/or cells on health and fertility of offspring and future generations, it is crucial that research provides new strategies to avoid irreversible harmful effects of metabolic imbalance during the periconception period. This is also important when using assisted reproduction techniques as they may create an unfavorable metabolic environment which can be challenging for the oocyte and the young embryo under *in vitro* conditions. On the other hand, representative *in vitro* systems are good tools to study the effect of metabolic stressors during different developmental periods, even if these production systems never provide the complete picture of an *in vivo* condition. The fact that *in vitro* models or embryo production systems often use oocytes from small-to-medium follicles of unknown metabolic background and that the time of maturation is hastened compared with the *in vivo* situation [15,16], may engender major bias when drawing conclusions from *in vitro* studies. As for other metabolic hormones and/or mediators, the cell response to insulin differs because of a variation in sensitivity, which depends on the metabolic background and the time point and duration of exposure. A more specific feature with insulin is its short biological half life and fast degradation. Insulin has been reported as very unstable in media containing cysteine [17], and this may justify the need to use higher dosages *in vitro* while making attempts to mimic the *in vivo* situation. As a result, the importance of a careful choice of adequate concentrations and exposure times to metabolic substances in experimental trials and in *in vitro* production systems becomes obvious. However, models in which insulin exposure differs from the *in vivo* situation may help to decipher cellular mechanisms influenced by insulin during oocyte maturation and early stages of embryonic development and to understand some of the dysregulations occurring *in vivo* because of high variation in circulating insulin levels.