Ovarian cysts in cows

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Anovulatory follicles and ovarian cysts occur in most animal species. The aetiology is multifactorial and the anovulatory follicles disturb reproduction in a species-specific manner. In cattle, and particularly in high-producing dairy cows, cystic ovarian follicles are frequently found in the early postpartum period with a reported incidence of 2-20%. Although most of the cysts self heal before the start of the insemination period but they may - if they persist - have great economic impact. Each incidence of ovarian cyst increases the calving interval and delays lactation, and infertile cows are at a greater risk of being slaughtered. Such unplanned, involuntary slaughter is very costly for the dairy farmer.

In early lactation, in the first weeks of the postpartum period, all cows are more or less in negative energy balance. This is accompanied by an initial insulin-resistant state in adipose and skeletal muscle tissues when nutrients are spared for the mammary gland's milk production. During the period of negative energy balance, there is a decrease in blood glucose, insulin and IGF-1 levels. The cow has to rely on tissue mobilization and fatty acids as energy source, and there will be increased circulating concentrations of non-esterified fatty acids and ketone bodies.

It is generally accepted that metabolic hormones and substances interfere with reproductive functions and that ovarian cysts develop due to a dysfunction in the hypothalamic-pituitary-gonadal axis. Sometimes the primary defect lies on the level of hypothalamus or the pituitary gland, affecting the luteinizing hormone (LH) release and thereby the final follicular growth and development. The preovulatory LH surge may be absent of insufficient, or occurring at the wrong time and not in synchrony with the maturing follicle. The reason is not too low GnRH content in the hypothalamus or reduced numbers of GnRH receptors or LH content in the pituitary but rather an altered feedback mechanism of oestrogen on the hypothalamus and pituitary. When the primary defect lies within the ovary or the follicle, the receptor expression in cystic follicle walls differ from normal follicles. LH receptors are less expressed in cysts, as well as and ER beta receptors that precede the increased expression of LH receptors. There is increased steroidogenesis in cysts, and higher expression of steroidogenic enzyme in oestrogen-producing cysts. Emerging cysts have decreased cell proliferation and increased apoptosis in granulosa and theca cells disturbing follicular growth and steroidogenesis.

Ovarian cysts, either oestrogen-producing follicular or progesterone-producing luteal cysts, are clinically diagnosed by rectal palpation and ultrasound combined with milk progesterone analysis, and treated with GnRH, prostaglandins or progesterone. The goal is to regain ovarian cyclicity by creating luteal structures which can be regressed by endogenous or exogenous PGF2α. More importantly, the formation of ovarian cysts can be prevented by proper management and nutrition of the transition cow (i.e. during late pregnancy, parturition and early lactation), and by selective breeding. By doing so, Swedish dairy farmers have successfully managed to decrease the treatment incidence from over 10% to 0.5% during the last 50 years. In conclusion, incidence of ovarian cysts and other fertility disorders can be considered good indicators of herd health and animal welfare.

Metabolic impact during pre-implantation and on the early embryo – IVF in humans

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During the last years increased concern has been raised for possible epigenetic effects on the gametes and embryos during assisted reproduction. There are several publications suggesting a possible association between assisted reproduction techniques (ART) and specific imprinting disorders. As these cases are very rare, and they occur in increased frequency also in subfertile couples, that have not been subject to ART, it is currently unclear how much is the impact of the ART procedure per se. There is however biological plausibility for this concern as mouse models have shown effects of embryo culture on gene imprinting. Could such rare cases be the tip of the iceberg, with more subtle epigenetic changes taking place in a larger proportion of the offspring after ART? The IVF/ICSI procedure includes several components that may interfere with epigenetic mechanisms. Generally, follow-up studies of children born after ART are reassuring with health profiles similar to those of the general population, the major concern being the high frequency of multiple pregnancies. Still, large national follow-up studies conclude that there is a slightly increased frequency of low birth weight children after ART, which is not entirely explained by subfertility, age or other confounding factors. Generally, mean birth weights are higher in children born after cryopreservation of the embryos. One study showed differences in mean birth weights for children born after ART with embryo culture in two different culture media. A recent Swedish national follow-up study showed a slight but significant increase in malformations and premature deliveries after prolonged embryo culture to the blastocyst stage compared with transfer at the earlier cleavage-stage. Reducing the oxygen content in the incubators to reach a composition closer to what is the physiological condition in the fallopian tubes seems to result in improved pregnancy frequency, but the effects on the offspring are yet unknown. Apart from possible effects of the ART procedure itself, the metabolic status of the female (and the male) counterpart could affect the outcome. Over-weight women experience an increased miscarriage rate, an increase that is also reported for recipients of donated embryos. Thus, the possibilities for metabolic and epigenetic effects in ART are numerous and complex, and the topic is now being studied in a large prospective study in Stockholm-Uppsala.

Metabolic impact during pre-implantation and on the early embryo in farm animals: *in vivo* and *in vitro* perspectives

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Growing awareness of maternal dietary / metabolic status implications plus advances in assisted reproductive technologies, whether aiming to complement or to transcend conventional livestock breeding options, have led to some of the most remarkable achievements and rewarding insights in the field of livestock reproductive biology in recent decades. In so doing they have extended our horizons of understanding in various dimensions, among these (i) new awareness of what can be achieved technically, (ii) appreciation of the longer time-frame within which an individual's life-long developmental capability is initially established and ultimately realized or undermined, and (iii) maybe most overlooked but not least important, the implications of 'pre-conception onwards' dam / donor / recipient status for offspring (and sometimes F2 generation) growth, health and reproductive competence in turn. Our impressions of the benefits and values, or otherwise, of technologies such as *in vitro* embryo production and nuclear transfer are rightly influenced by the extent to which they impinge on the health of animals either subjected to or derived from them. Dietary and related (e.g. endocrine and metabolic) effects should be no less carefully scrutinised. Lessons learned that may be applicable elsewhere (for instance, now apparent but initially unexpected parallels between metabolism of pre-implantation embryos and that of cancer cells) also could be rewarding in unexpected ways. In passing judgement on an embryo's metabolic response, whether *in vivo* or *in vitro*, to particular conditions, we also must remain open-minded about what constitutes a 'norm' in atypical conditions. Transient deviations from expected metabolic parameter ranges may not always be indicative of aberrant development but instead could be, at least occasionally, a benign adaptation indicative of resilience. As evidenced by data supporting the 'quiet embryo' hypothesis, busiest isn't always best metabolism-wise (and may in fact be indicative a burnout event) so we continue to learn lessons from life at its earliest stages. The marvel, after all, is that successful development is so often achieved!

Gestational diabetes in the dog

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Through a variety of mechanisms, pregnancy causes insulin resistance, which suppresses the cellular uptake of glucose and increases the demands on the beta cell. In the extreme, gestational diabetes (GDM) mellitus may develop. There are few reports on the clinical appearance, prognosis, and risk factors for GDM in dogs. In opposition to the human situation, pregnant dogs are not generally screened for impaired glucose tolerance. We aimed to describe the clinical characteristics of GDM in dogs, through at retrospective study of thirteen dogs with GDM. Medical records were reviewed and owners and referring veterinarians were contacted for follow-up information. We found that Nordic Spitz breeds (11/13 dogs) were overrepresented in the case material. Diagnosis was established at a median of 50 days after mating (range, 32-64). Median glucose concentration at diagnosis was 18.9 mmol/L (range, 11.3-32.0). One dog was euthanized at diagnosis, 5 bitches were treated with insulin until whelping, and in 7 dogs, pregnancy was terminated within 4 days of diagnosis. One dog died after surgery. Tight glycemic control was not achieved in any of the insulin-treated dogs during pregnancy. Diabetes mellitus (DM) resolved in 7 dogs at a median of 9 days after the end of their pregnancies and DM was permanent in 4 dogs. Puppy mortality was increased compared with offspring of healthy dams. This report suggested that GDM affects mainly middle-aged bitches in the 2nd half of pregnancy with a breed predisposition toward Nordic Spitz breeds. GDM may resolve within days to weeks after pregnancy has ended. Further research is needed to investigate optimal treatment regimens for dogs with GDM and risk factors for unsuccessful outcome.

Metabolic short and long term effects in the offspring after starvation or diabetes/obesitas in the mother

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Being born small, large or too early, all put the individual at risk of developing metabolic consequences both in the short and long run.

During fetal life infants born small for gestational age have often suffered of a lack of nutrients and oxygen. The adaptations to these relationships are not always adequate after being born. The newborn infant has too small energy depots of glucose and fat to sustain the first few days of starvation before the breast milk of the mother is available.

During this period there is an increased risk of hypoglycemia, hypothermia and other complications as infections. Later in life epidemiological studies have shown that these infants have an increased risk of cardiovascular and metabolic disease. Studies in animal models have shown a relationship between intrauterine growth restriction and development of a reduced vascular bed and number of nephrons in the kidneys and a thinner aortic width and stiffer aortic wall, resulting in increased blood pressure. The growth of the pancreas is also attenuated resulting in fewer islands of Langerhans and reduced capacity of insulin secretion later in life. All these changes lead to an increased risk of developing cardiovascular disease and type 2 diabetes mellitus. Studies in children and adolescents being born small for gestational age show early signs of high blood pressure and impaired glucose tolerance, confirming the animal data.

Infants being born preterm also have small energy depots and add the risk of hypoglycemia and hypothermia to all the other complications of being born early. Preterm infants are often malnourished during the first weeks of life, due to difficulties to safely mimic the delivery of nutrients during fetal life. A state of “extra-uterine” growth restriction develops. Follow-up studies in these infants show that they develop the same pre-stages of both high blood pressure and type 2 diabetes mellitus as infants with intrauterine growth restriction.

During diabetic pregnancy the fetus has a surplus of glucose and other energy substrates in the circulation. High glucose and amino acid levels stimulate fetal insulin and IGF-I secretion, resulting in increased growth of the fetus – macrosomia. There is a 5 to 10-fold increased risk of having an infant born large for gestational age after diabetic pregnancy. After birth the energy transport via the umbilical cord is discontinued and there is a risk of hypoglycemia due to hypersecretion of insulin. These infants need immediate regular feeds and close monitoring of blood glucose levels the first days of life, when changing over to intermittent breast feeding.

A surplus of energy during fetal life also puts the infant at risk of developing cardiovascular and metabolic disease later in life. Studies of high-risk populations of developing type 2 diabetes mellitus, like the Pima Indians in Arizona, USA, have shown that there is both a genetic predisposition and also a metabolic effect on the fetus of high glucose levels increasing the risk of metabolic syndrome later in life. This metabolic impact might have its effect via epigenetic changes that can be forwarded over generations.

Infants born small, large and preterm share a common risk of developing cardiovascular and metabolic disease later in life, but the pathogenesis differs.

Starvation or diabetes/obesitas in the mother

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Characterization of fetal outcome in pregnancies with different maternal metabolic status and, consequently, with different modes of nutritional supply to the embryo/fetus. Pathogenesis and etiological mechanisms of embryo-fetal developmental disturbances when the pregnant woman suffers from starvation, diabetes mellitus or obesity.