

Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review

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Abstract – Cystic ovarian follicles (COF) are an important ovarian dysfunction and a major cause of reproductive failure in dairy cattle. Due to the complexity of the disorder and the heterogeneity of the clinical signs, a clear definition is lacking. A follicle becomes cystic when it fails to ovulate and persists on the ovary. Despite an abundance of literature on the subject, the exact pathogenesis of COF is unclear. It is generally accepted that disruption of the hypothalamo-pituitary-gonadal axis, by endogenous and/or exogenous factors, causes cyst formation. Secretion of GnRH/LH from the hypothalamus-pituitary is aberrant, which is attributed to insensitivity of the hypothalamus-pituitary to the positive feedback effect of oestrogens. In addition, several factors can influence GnRH/LH release at the hypothalamo-pituitary level. At the ovarian level, cellular and molecular changes in the growing follicle may contribute to anovulation and cyst formation, but studying follicular changes prior to cyst formation remains extremely difficult. Differences in receptor expression between COF and dominant follicles may be an indication of the pathways involved in cyst formation. The genotypic and phenotypic link of COF with milk yield may be attributed to negative energy balance and the associated metabolic and hormonal adaptations. Altered metabolite and hormone concentrations may influence follicle growth and cyst development, both at the level of the hypothalamus-pituitary and the ovarian level.

cystic ovarian follicles / pathogenesis / hypothalamus-pituitary / ovary / negative energy balance

1. INTRODUCTION

Cystic ovarian follicles (COF) are an important cause of subfertility in dairy cattle, since they extend the calving interval [1–3]. Prolongation of the calving interval and treatment costs of COF result in economic loss for the dairy farmer. In most of the literature, COF are referred to as Cystic Ovarian Disease (COD). However, this terminology should be revised since the emphasis on cystic follicles has shifted over time.

In the 1940's, the presence of cystic follicles on the ovaries was mainly associated with nymphomania and a bull-like appearance in cows [4, 5], which are clear clinical signs of a state of "disease". Over the past decades, dairy herd management and economics have evolved to a situation in which fertility in the postpartum period is utterly important. During this period, cystic follicles are rather common, and generally occur without obvious clinical signs. Normal ovarian cyclicity is, however, delayed and

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these cysts should therefore be regarded as COD, despite the absence of previously “classical” signs of disease in the majority of cases. In addition, after a variable period of time, cysts can become non-steroidogenic and then they no longer interfere with cyclicity [6, 7]. Consequently, at the time the non-steroidogenic cyst is observed, no other clinical abnormalities are present.

In present-day dairy herd health programmes, “cysts” are often diagnosed in the absence of clear clinical signs. Therefore the term “Cystic Ovarian Disease” no longer seems appropriate and should be replaced by the term “Cystic Ovarian Follicle(s)” which does not necessarily implicate a state of disease. In this review, we will therefore use COF instead of COD. We prefer to use COF instead of “ovarian cysts”, because the former term indicates that it is the ovarian follicle(s) and not any other ovarian tissue that becomes cystic.

2. DEFINITION

Cystic ovarian follicles develop when one or more follicles fail to ovulate and subsequently do not regress but maintain growth and steroidogenesis. They are defined as follicle-like structures, present on one or both ovaries, with a diameter of at least 2.5 cm for a minimum of ten days in the absence of luteal tissue [8–11]. It has become clear though that this definition needs to be revised. First, the size limit is rather artificial since follicles might already become cystic at a smaller size, and dominant follicles ovulate on average at a size of 1.6 to 1.9 cm in dairy cows [12–14]. Moreover, many researchers showed that COF are actually dynamic structures, which can regress and be replaced by new cysts [15–18]. The factors that determine whether a cyst will regress or not, remain unknown [19, 20], although changes in mean LH concentration seem to be involved [16]. So the required individual persistency of ten days is questionable. In addition, in practice, veterinarians generally do not have the oppor-

tunity to perform a second examination of an animal ten days after the initial diagnosis of COF to fulfill all the terms of the definition. The absence of a corpus luteum is another requirement, which is not always fulfilled [21]. Non-steroidogenic cysts which are hormonally inactive do not influence the normal oestrous cycle, so they can occur together with a corpus luteum. Therefore, recent research articles define COF differently and perhaps more logically [16, 22–24], although a generally accepted definition is still lacking, which can also be attributed to the heterogeneity (type of cyst, time of occurrence, clinical signs) of the cysts.

Based on the current knowledge and recent literature, COF may be defined as follicles with a diameter of at least 2 cm that are present on one or both ovaries in the absence of any active luteal tissue and that clearly interfere with normal ovarian cyclicity.

Macroscopically, cysts can be subdivided into follicular and luteal cysts, which are considered to be different forms of the same disorder [25]. Luteal cysts are believed to be follicular cysts in later stages [26]. Determination of progesterone concentrations in blood plasma, milk or milk fat can help to make a distinction between the two types. Follicular cysts secrete little or no progesterone while luteal cysts clearly do [26]. However, the threshold values used in the literature differ a lot [27–31], which makes it difficult to set a concentration threshold. In addition, the many intermediate forms with limited or extensive luteinisation do not allow for a clear identification of cyst type. So classification is not easy and is subject to personal interpretation. Ultrasound can be useful in supplying extra information. Follicular cysts have a thin wall (≤ 3 mm) and the follicular fluid is uniformly anechogenic, while luteal cysts have a thicker wall (> 3 mm), which is visible as an echogenic rim. Also, the latter often have echogenic spots and web-like structures in the follicular fluid [32, 33]. Luteal cysts should not be confused with hollow corpora lutea, which are not pathological at all [26].

Hollow corpora lutea are just young corpora lutea with an antrum [34]. Ultrasound examination of the ovaries is useful in making a distinction between a luteal cyst and a cystic corpus luteum [32, 35].

Follicular cysts initially continue to produce oestrogens in the absence of other follicles > 5 mm on ultrasound [36]. After a variable period of time oestrogen production may cease. The cyst becomes non-steroidogenic without luteinising, thereby allowing a new follicular wave to emerge and follicles to grow beyond 5 mm [6, 7].

3. INCIDENCE AND SIGNS

Cystic ovarian follicles can occur at different times throughout lactation. The incidence varies between 6 and 30% [9, 11, 37–43]. The diagnosis of COF is most often made during the first 60 days post partum [8, 9, 38, 44], mainly because of the close monitoring of cow fertility during this period. The majority of all cysts occur throughout this stage [37, 40, 43]. The self-recovery percentage of these early cysts is 60–65% [8, 9, 38, 43]. Despite this high self-recovery rate, the importance in dairy cow fertility is considerable [45]. As reported by Thatcher and Wilcox [46] and more recently by Shrestha et al. [47], early resumption of ovarian cyclicity is beneficial for fertility. By delaying/interfering with ovarian cyclicity, COF increase the time to first insemination and the interval from parturition to conception. In addition, COF decrease the pregnancy rate after first insemination and increase the number of services per conception [47, 48].

A genetic predisposition exists for COF [49, 50], but the heritability is rather low, being 0.07 to 0.12 [51–53]. However, the incidence in Dutch Holstein Friesian herds is actually increasing [51]). Genetic selection to reduce the incidence of COF can be successful, despite the low heritability [54].

The clinical signs that accompany ovarian cysts are variable. Anoestrus is most

common, especially during the postpartum period [9]. Irregular oestrus intervals, nymphomania, relaxation of the broad pelvic ligaments and development of masculine physical traits are other signs which may be present, especially later during lactation [11, 55].

4. PATHOGENESIS OF OVARIAN CYSTS

Ovarian dysfunctions like cysts occur most often during the early postpartum period when there is a transition from the non-cyclic condition during pregnancy to the establishment of regular cyclicity. It is generally accepted that cystic follicles develop due to a dysfunction of the hypothalamic-pituitary-ovarian axis. This dysfunction has a multifactorial etiology, in which genetic, phenotypic and environmental factors are involved [9, 19, 26]. When discussing the pathogenesis of COF, a distinction may be made between a primary defect in the hypothalamus-pituitary and a primary defect at the level of the ovary in the follicle itself. However, COF formation may result from defects in both ovary/follicle and the hypothalamus/pituitary as well.

4.1. Hypothalamic-pituitary dysfunction

The most widely accepted hypothesis explaining the formation of a cyst is that LH release from the hypothalamus-pituitary is altered: the pre-ovulatory LH-surge is either absent, insufficient in magnitude or occurs at the wrong time during dominant follicle maturation, which leads to cyst formation [8, 16, 18, 41] (Fig. 1). This aberrant LH release does not seem to be caused by a lower GnRH content of the hypothalamus, nor by reduced GnRH receptor numbers or LH content in the pituitary [56, 57].

It is believed that an altered feedback mechanism of oestrogens on the hypothalamus-pituitary can result in an aberrant

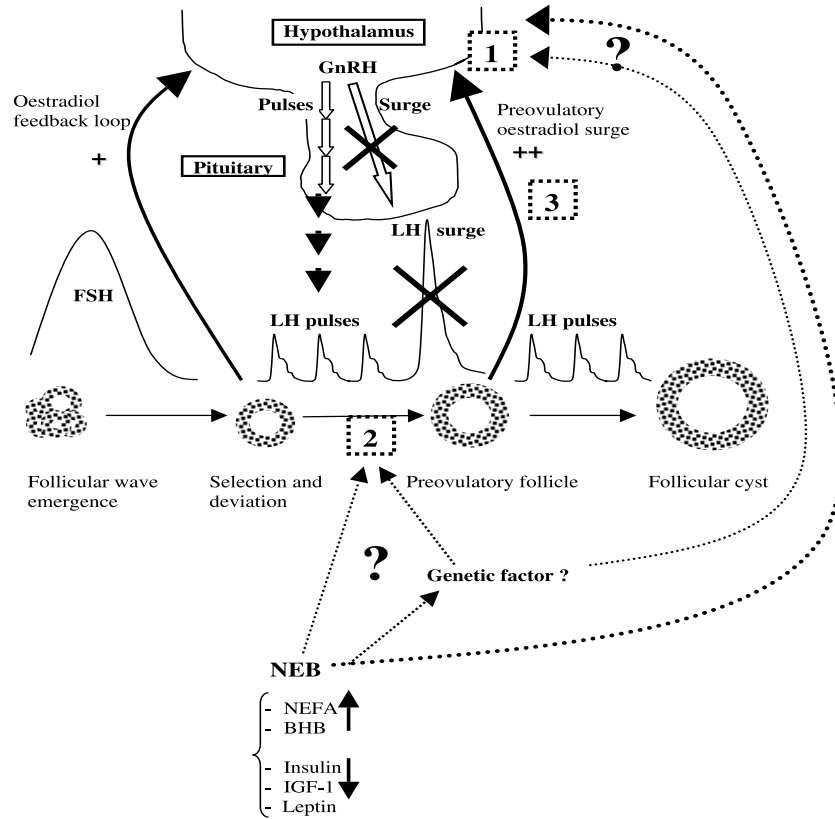


Figure 1. Schematic representation of the pathogenesis of ovarian cysts and the possible pathways involved. An FSH surge stimulates the emergence of a new follicular wave, from which a single dominant follicle is selected at the time of deviation. Through a positive feedback loop oestradiol stimulates GnRH and LH pulsatility, which in turn supports growth and development of the dominant follicle. Upon reaching preovulatory size, follicular steroidogenic activity reaches a peak and produces a preovulatory oestradiol surge. This surge either fails to elicit a GnRH and subsequent LH surge or the GnRH/LH surge is mistimed/delayed. The dominant follicle, therefore, does not ovulate but, due to the ongoing LH pulsatility, continues to grow and becomes a cyst. The disruption of the hypothalamic-pituitary-gonadal axis can be caused by factors affecting the oestradiol feedback mechanism and GnRH/LH release at the hypothalamic-pituitary level (1) and/or by an aberrant follicle growth and development with alterations in receptor expression and steroidogenesis (2), leading to an altered oestradiol surge and feedback (3). Hypothalamic-pituitary function and follicular growth/development may be affected by NEB through metabolic/hormonal adaptations. In addition, in the situation of NEB, the expression of genetic hereditary factor(s) associated with COF may be promoted or the functional importance may increase, which in turn may affect follicle growth and hypothalamic-pituitary function.

GnRH/LH release and cyst formation. A GnRH/LH surge occurring prematurely during follicle growth, i.e. when no follicle capable of ovulation is present, can render the hypothalamus unresponsive to the feedback effect of oestradiol which results in the

formation of ovarian cysts [22, 58]. To restore the feedback mechanism, the hypothalamus needs to be exposed to progesterone [59, 60]. Consequently, a similar state of hypothalamic refractoriness to oestrogens and subsequent cyst formation can be achieved if

the progesterone rise after a spontaneous ovulation is prevented [61]. This physical state of hypothalamic unresponsiveness to oestrogens seems to be present in the majority of cows with COF, as illustrated by the failure of an exogenous oestradiol treatment to elicit a timely LH surge [62–65]. However, the refractoriness of the hypothalamus-pituitary for oestradiol in cows with COF may be a consequence rather than a cause of the disease. Removal of the cystic ovary by ovariectomy restores the feedback mechanism and the capacity of oestradiol to elicit an LH surge, although the underlying mechanism is not known [66].

An altered feedback mechanism and GnRH/LH release may be attributed to factors interfering at the hypothalamic-pituitary level. Progesterone at suprabasal concentrations blocks the LH-surge, thereby inhibiting ovulation, but increases the LH pulse frequency [67, 68]. This results in an anovulatory, persistent follicle with a larger diameter and a longer lifespan than normal, and increased peripheral oestradiol concentrations [68]. These follicular and hormonal changes are very similar to observations made in cows with COF [16]. Recently, Hatler et al. [23] observed that at the time of diagnosis, most cysts are accompanied by suprabasal progesterone concentrations, which play a role in cyst turnover. These observations together with the similarities between persistent follicles, induced by suprabasal progesterone, and naturally occurring cysts, suggest a role for progesterone in the pathogenesis of COF. However, suprabasal progesterone profiles seem to play a limited role in primary COF formation [69]. Factors indirectly reducing GnRH/LH secretion like stress [6, 70–72], intrauterine infections [44, 73] and seasonality [74] are also considered to increase the risk of cyst formation.

In cystic cows, the formation of new cysts is accompanied by increased LH pulse frequencies and amplitudes [16, 57]. However hypersecretion of LH does not seem to be involved in cyst formation, but it may play a role in cyst persistence [75]. Data obtained

in sheep also dismiss an increased LH secretion as a primary cause of COF [76].

In conclusion, an aberrant LH surge is likely to be the trigger for the development of COF. Abnormal LH release seems to be caused by an altered feedback mechanism of oestrogens on the hypothalamus-pituitary. The malfunctioning of the feedback mechanism can be caused by factors directly interfering at the hypothalamic-pituitary level or by an altered follicle growth and development disrupting the hypothalamic-pituitary-gonadal axis, as discussed below.

4.2. Ovarian/follicular dysfunction

A primary dysfunction at the level of the follicle may disrupt the hypothalamic-pituitary-ovarian axis and cause the formation of COF (Fig. 1). First of all, alterations in LH receptor expression and content may cause anovulation of the follicle. The LH surge initiates a complex multi-gene, multi-step process in which timing is essential, finally leading to ovulation of the pre-ovulatory follicle [77]. According to Kawate et al. [78], FSH and LH receptor numbers in granulosa cells of cysts are decreased when compared to normal follicles, but this is contradicted by data from Odore et al. [79] and Calder et al. [80]. Discrepancies between studies may be explained by differences in methodology such as demonstration of the receptor itself or its mRNA, and the division of cysts into oestrogen-active and oestrogen-inactive. In the same study, Calder et al. [80] also studied developing “young cysts” but no differences in FSH/LH receptor mRNA were observed when compared to dominant follicles. Young cysts were, however, studied in the presence of existing cysts, i.e. when the endocrine environment was already altered, and therefore the pathogenesis may differ from primary developing cysts.

Another receptor of interest is the oestradiol receptor β (ER- β). In rodents, the importance of this receptor in follicular growth and development has clearly been demonstrated

[81, 82] and its localisation in follicle cells throughout follicular development has been described in many mammals including cattle [83, 84]. More specifically, in rat ovarian follicles ER- β mRNA expression precedes increased expression of mRNA for the LH receptor and specific steroidogenic enzymes [85]. Therefore, alterations in ER- β expression may be involved in the development of COF. However, this hypothesis is not supported by data from Calder et al. [80] showing that ER- β mRNA expression was not altered in growing young cysts. Odore et al. [79] did, however, find decreased oestrogen receptor concentrations in follicular cysts, but the oestrogen receptor type was not defined.

Besides changes in receptor expression and content, alterations in steroidogenesis by the dominant follicle may also be involved in cystic degeneration. After all, the dominant follicle has to elicit an LH surge at the right time in its development by producing sufficient oestradiol. Oestrogen-active cysts show a higher expression of 3 β -hydroxysteroid dehydrogenase mRNA, a steroidogenic enzyme [80], and cows developing a cyst have increased oestradiol concentrations during the early stages of follicular dominance [86]. However, Calder et al. [80] were unable to observe changes in mRNA expression of steroidogenic enzymes in the follicle wall of young growing cysts. They concluded that alterations of the endocrine system precede, and perhaps cause, the observed follicular alterations in cysts. In the study of Calder et al. [80], young cysts did, however, develop in the presence of existing cysts, i.e. when the endocrine environment was already altered. As a consequence, the mechanism causing these "young cysts" to actually become cysts may differ from the mechanism(s) involved in primary cyst formation.

Apart from changes in mRNA expression for certain receptors and steroidogenic enzymes, cell proliferation and apoptosis in the granulosa and theca interna cell layers also seem to be altered in cystic follicles.

Early cystic follicles show an increase in apoptosis while cell proliferation is decreased [87, 88]. Although it is hard to establish a cause-effect relationship, alterations like these may disrupt normal follicle growth and steroidogenesis leading to cystic degeneration.

Recently, Imai et al. [89] suggested that matrix metalloproteinases (MMP) could be involved in the formation of cysts: higher proMMP-2 and -9 levels were present in the follicular fluid of cysts than in the follicular fluid of normal dominant follicles. MMP play a role in follicle wall remodelling and rupture at the time of ovulation [77, 90], but hereto the inactive proMMP form needs to be transformed to the active MMP form. This activation is triggered by the LH-surge [77]. Since an aberrant LH-surge causes COF formation, the higher proMMP-2 and -9 levels in the follicular fluid of COF are most likely an indication of the lack of an LH-surge rather than a cause of COF formation.

4.3. Predisposing factors for COF

As mentioned before, COF are mainly observed in high yielding dairy cows during the first months post partum and milk yield is generally considered a risk factor [40, 51, 88, 86, 91–93], although not all authors agree [37, 94]. Moreover, besides the fact that a genetic predisposition for COF exists (see above), a genetic correlation between cysts and milk production traits has been established, indicating that an ongoing selection for production parameters will increase the incidence of COF [51]. What the genetic factor(s) are and how they promote the formation of cysts is not known. However, the fact that cows do not develop a cyst during every lactation and during every ovarian cycle indicates that gene expression may be promoted by, or gains functional importance under, certain stressors, for example high milk yield and the associated negative energy balance (NEB) during the early postpartum period. At this

time, energy requirements to sustain milk yield are higher than energy intake thus causing a NEB. This NEB is accompanied by several hormonal and metabolic adaptations, affecting ovarian function [95]. Energy balance may be a more accurate parameter than milk yield to further elucidate the association between COF and production traits. Some animals can compensate for higher milk production through greater dry matter intake reducing the effect of milk yield on energy balance [92]. This could explain why not all authors [37, 94] observed a correlation between ovarian cysts and milk yield. However, when focussing on energy balance and the occurrence of COF, the results still remain inconclusive. While Zulu et al. [24], Refsdal [43] and Sovani et al. [96] observed a deeper NEB and increased mobilization of body reserves in cows developing cysts, Beam [86] noticed that the nadir of the NEB occurred later post partum in cystic cows than in ovulatory cows. Moreover, cystic cows even mobilized less body reserves and derived a smaller percentage of their milk yield from body weight loss [86]. Hooijer et al. [97] were unable to find a more severe NEB, evaluated by the fat/protein ratio in milk, in cows with COF compared to ovulatory cows. However in an earlier study, Heuer et al. [91] observed that a high fat/protein ratio, and, therefore, a more severe NEB, increased the risk of cyst occurrence. Data in sheep also suggest that an increased mobilisation of body reserves, indicative for a deeper NEB, is linked with the occurrence of cystic follicles [76]. Although a consensus is lacking, we conclude from the literature that a link seems to exist between COF and the magnitude and/or duration of the NEB.

The possible underlying mechanism(s) is(are) also still unclear, but NEB may affect COF formation at both the level of the hypothalamus/pituitary and the ovary/follicle through associated hormonal and metabolic changes [98, 99] (Fig. 1). During NEB, peripheral plasma concentrations of IGF-1, insulin, glucose [95] and leptin [100, 101] are reduced, while concentrations of metab-

olites such as non-esterified fatty acids (NEFA) [102] and β -hydroxybutyrate (BHB) are increased [103]. The IGF-system plays an important role in follicle growth and development [104]. Besides a direct effect, IGF-1 together with insulin indirectly stimulates follicular development through upregulation of the LH-receptor on granulosa cells [105]. Therefore, low systemic IGF-1 concentrations early post partum could contribute to anovulation and subsequent development of cystic follicles as shown by Zulu et al. [24]. However, data from Beam [86] do not confirm this hypothesis. Also insulin itself is known to be a potent stimulator of follicle cell steroidogenesis and proliferation in vitro [106, 107] and in vivo [108–110]. Consequently, reduced circulating insulin concentrations early post partum may play a role in ovarian dysfunction i.e. cyst formation, as we have recently demonstrated [69]. Besides low insulin concentrations, a general state of peripheral insulin resistance is present as well in high yielding dairy cows early post partum [111, 112]. Insulin resistance is regarded as an important factor in the pathogenesis of the Polycystic Ovary Syndrome (PCOS) in women [113–115] and COF have often been compared to this syndrome, justified or not. However, insulin insufficiency rather than insulin resistance has been observed in COF cows [116], indicating an altered interaction between glucose and insulin at the pancreatic level. In addition, in ewes it was not possible to induce cyst formation through the establishment of a state of insulin resistance [76]. Conclusively, IGF-1 and insulin are important stimulators of follicle growth and based on the limited number of publications on the subject, low concentrations of one or both hormones may contribute to the formation of COF (Fig. 2). Further research should confirm whether or not this hypothesis is valid.

Leptin is a recently “new” hormone, produced by adipocytes, and is regarded as the ultimate factor linking metabolic status to reproduction [117]. Depending on the metabolic state of the animal it either has a

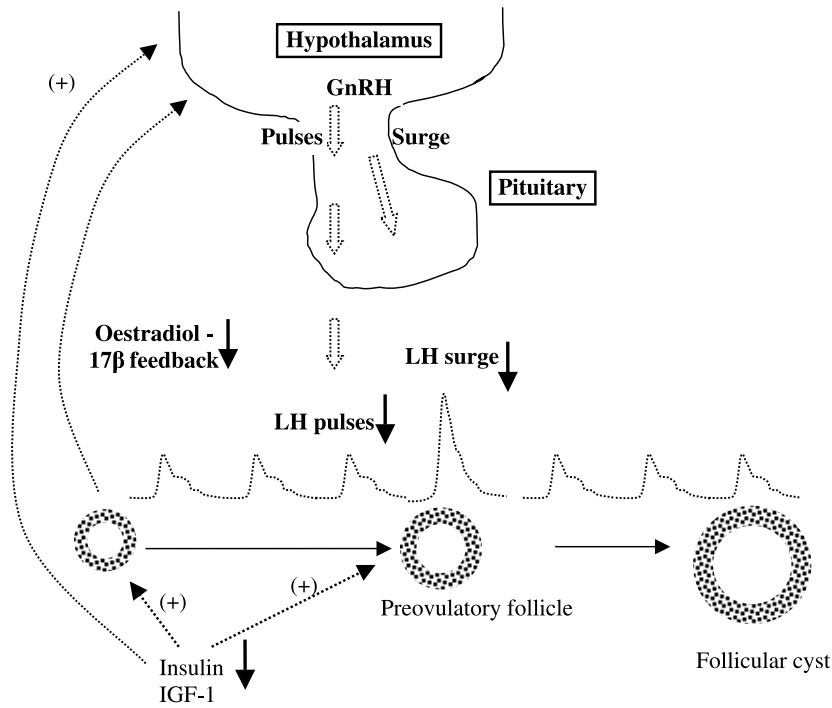


Figure 2. Schematic model of how low insulin and/or IGF-1 concentrations may cause cyst formation. Low insulin/IGF-1 concentrations insufficiently (+) stimulate follicle cell proliferation and oestradiol-17 β production. The reduced oestradiol-17 β feedback, together with the low insulin/IGF-1 concentrations result in a reduced gonadotropin release. Dominant follicle growth is retarded and the altered follicular growth pattern and oestradiol-17 β production disrupt the hypothalamo-pituitary-gonadal axis. This finally results in an aberrant LH-surge and the subsequent development of a cystic follicle.

stimulatory effect or none at all on hypothalamic-pituitary function in cattle [118–120]. In the postpartum dairy cow, a clear relationship between leptin profiles and first postpartum ovulation is lacking [101], although a minimum permissive level of leptin seems required to induce the first postpartum LH surge [101, 121]. Therefore, leptin may play a role in early post partum cyst development.

According to Zulu et al. [24] and Huszenicza et al. [122] cows developing cysts have higher serum NEFA concentrations during the first week(s) post partum than ovulatory cows, although Beam [86] was unable to observe this. Interestingly, in rats, elevated NEFA concentrations for

48 h can decrease insulin secretion by the β -cells of the pancreatic islets in response to a glucose challenge [123]. Moreover, NEFA are cytotoxic for several cell types [124–127], including bovine granulosa and theca cells [128, 129]. So (prolonged) exposure to high NEFA concentrations during periods of NEB may hamper follicle growth and development, disrupting the complex endocrine system and promoting the formation of ovarian cysts.

Although elevated serum ketone concentrations increase the risk of delayed cyclicity [122, 130, 131] and cyst occurrence [132, 133] post partum, they do not exert any negative effects on bovine follicle cells in vitro [134]. Consequently, ketone concentrations

in the postpartum dairy cow seem to be an indicator of the severity of the NEB, but not a mediator of the negative effects of the NEB on reproduction at the ovarian level.

5. CONCLUSION

Cystic ovarian follicles are one of the most frequent and important ovarian disorders in modern high yielding dairy cows that have been the subject of much research in recent decades. However, many aspects of the disease, and especially pathogenesis, remain unclear and inconclusive, as for example, illustrated by the lack of a clear definition. In particular, the endocrine and follicular changes that precede spontaneous cyst formation are still unknown, mainly due to the heterogeneity and unpredictability of the disease. Studies aimed at elucidating the pathogenesis, have tried to do so by induction of cysts. This, however, may not mimic naturally-occurring cysts. Nevertheless, such experiments have enhanced our knowledge about the endocrine and follicular changes that occur after cyst formation. Development of an accurate model mimicking the *in vivo* situation or identification of criteria to allow classification of a follicle as a future cyst before it actually becomes cystic, would be very valuable in studying the cellular and molecular changes that precede ovarian cyst formation.

Due to the genetic correlation with production traits and the high incidence of ovarian cysts during the period of NEB early post partum, future research should also focus on the effect of NEB-associated metabolic/hormonal changes and energy utilisation on follicular development and steroidogenesis. Understanding how NEB affects cyst formation will help to optimise management and feeding practices in preventing the occurrence of ovarian cysts/COF.

Further research on cellular changes in follicular cysts may elucidate which genes show an altered expression pattern com-

pared to normal dominant follicles and could therefore be involved in the primary development. Genetic knock-out models as well may help to determine which genes play a role in cyst formation. The identification of these genes would be an initial step in the process of identifying the hereditary factor(s), making it possible to genetically screen bulls and cows for COF prior to their use in artificial insemination programmes.

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