

# **Reproduction in Domestic Animals**

**Physiology, Pathology, Biotechnology**

**Blackwell  
Science**

**2nd Hungarian Reproduction Meeting  
Balatonfüred, Hungary  
19–20 September, 1994**

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Dear Reader,



In 1993 we arranged the first Hungarian meeting on Animal Reproduction and all participants were convinced that it should be repeated soon. Of course each year there are already several international and national meetings on reproduction, but it seemed to be necessary to initiate such conference for the East European countries establishing personal contacts between our scientists and the colleagues from the Western countries.

This year's conference is related to swine reproduction and we are glad that some of the most sophisticated specialists from the Czech Republic, France, Germany, Italy, Poland, Sweden, UK and Hungary followed our invitation. In 16 lectures they will present data on embryology, ovarian physiology and its endocrine control, in vitro techniques, semen preservation and the importance of management, nutrition and prolificacy in different breeds. You may find some mini reviews and summaries of the presented data in this issue of "Reproduction in Domestic Animals".

The meeting <sup>was</sup> held in Balatonfüred, a beautiful old town at the Lake Balaton. We hope the participants <sup>kept</sup> will keep this conference and this place in good memory and the readers of this issue of Reproduction in Domestic Animals will get some new information as well.

We gratefully acknowledge the support from the official partner ROUSSEL UCLAF and Autopress-Korando, Bioconsult Co., Erdos Co., Hungarian Artificial Insemination Ltd., Hungarian Commercial Bank Ltd., Noack-Hungary, Phar-Accord (European Union), Pharmavet Co., Prim-A-Vet Co., Sanofi-Phylaxia, Uni-Vet. Co., Vet-Unitrade Co., Hungarian Ministry of Agriculture and all others who made this conference possible.

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## **Reproductive Management in Sows with Respect to Efficiency and Pig Welfare**

Smidt, D.

The efficiency of piglet production is largely dependent on the quality of reproductive management in sows. Management systems have considerable impact on the welfare of sows, boars and piglets. In this context, reproductive performance and welfare of pigs can be regarded as closely related criteria. Stress-situations imposed on pigs will, mainly via neuroendocrine and metabolic pathways, negatively interfere with reproductive functions as well as reduce the level of animals' well being. Environmental conditions, which do not comply with ethological and physiological requirements of pigs, will also impair reproductive efficiency. Therefore, reproductive management as well as the design of housing and pig facilities have to be focussed on 2 important goals, namely

- optimal reproductive efficiency and
- suitable conditions for pig welfare.

In this context, the most important elements and their interdependences can be characterized as follows:

1. High reproductive performance largely depends on optimal functional efficiency in boars, sows and piglets. The respective physiological requirements and prerequisites can only be realized by the employment of good management practice and proper design of housing and equipment.
2. Good management practice with reference to reproductive efficiency and pig welfare in different functional states mainly comprise the following aspects:
  - 2.1 The management of gilts aims at sexual maturity at a proper state of age and physical development, which meets the requirements of fertility as well as of welfare with respect to mating, parturition and rearing. Group housing of premature gilts and keeping them in a condition of physical fitness, are favourable prerequisites for respective achievements.
  - 2.2 Reproductive management of sows during estrus and mating/AI is expected to stimulate estrus behaviour, to optimize the ovulation rates and fertilization rates, and to allow for the fulfilment of behavioural requirements. This refers to sexually mature gilts as well as to sows in the postweaning period. Achievements are based on the utilization of stimulatory effects on estrus and ovulation rates in sows and reproductive capacity of boars in properly designed mating centres. Mating centres must allow for mutual olfactorial, auditive and, possibly, visual stimulatory contacts of gilts and postweaning sows with boars.

- 2.3 During pregnancy the individual food supply according to the functional state of sows and fetuses as well as the avoidance of severe stress situations contribute considerably to the achievement of high fertility rates and acceptable welfare levels in the management of pregnant sows. Recent technical developments, like transponder-feeding, electronic identification, computer-aided management, allow for group housing of pregnant sows, without neglecting the obvious advantages of single-keeping systems like individual care and feeding or easy handling and treatment of individual animals. Group housing, however, if organized properly, is more likely to meet the functional requirements for reproductive efficiency as well as for animal welfare. In pregnant sows, therefore, shifts towards group housing, can be observed.
- 2.4 The management of parturition aims at an easy course of births without obstetrical complications. This is very important for a low incidence of perinatal losses and for the postnatal viability of piglets. High weaning rates require housing conditions which comply optimally with ethological and physiological requirements of piglets and sows. The standard procedure is to keep sows in separate farrowing stables during parturition and suckling period. Many attempts are made to modify the conventional farrowing crates in order to have them more adapted to behavioural requirements of sows and piglets., e.g. with respect to the locomotion of sows and microclimatic conditions for piglets. First attempts with group housing of sows and piglets during the suckling period are also quite promising with respect to rearing efficiency and welfare. A further important goal during this period is a low incidence of peri- and postnatal diseases in sows, which may interfere with fertility.
- 2.5 Optimal weaning times and weaning procedures may increase the number of litters per year and thus contribute to the improvement of reproductive efficiency. Very early weaning, however, may cause fertility problems in sows and may interfere with welfare requirements.
- 2.6 The employment of biotechnical procedures suitable for the improvement of fertility, for the rationalization of reproductive processes as well as for functional diagnosis, e.g. ovulation or pregnancy will contribute to the improvement of reproductive performance.
3. All measures in reproductive management have to aim at optimal economic results in pig production, at the same time, however, at strict observation of pig welfare requirements. Therefore, welfare orientied care, supply and handling of sows, boars and piglets is an important element in successful pig breeding. This can be facilitated effectively by the employment of computer aided concepts of reproductive management.

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### **Relationship between Nutrition and Fertility**

Einarsson, S. and Tsuma, V.T.

#### **Introduction**

It has for long time been known that a relationship between nutrition and fertility exists, but the physiological mechanisms by which intake of nutrients affect fertility of the sow have not been clearly determined. The energy and protein substrates that are necessary for the physiological processes are derived from the diet and the body reserves. When dietary supply exceeds the demand of these processes, then substrates are stored as fat, glycogen or protein. When, on the other hand, the demand exceeds dietary intake, these reserves are mobilized. It has been suggested that the long-term reproduction of the sow is best served by minimizing weight and fat loss in lactation. Such a strategy would require only a minimal restoration of weight in the following pregnancy which would be beneficial since the greater feed intake and weight gain in pregnancy the greater the weight loss in lactation (from Einarsson & Rojkittikhun, 1993).

#### *Nutrition and embryo survival*

In some experiments a relationship has been shown between feed intake and embryo survival. Improved embryo survival with gilts given lower feed levels has been reported (e.g. Dyck & Strain, 1980). They demonstrated a benefit when feed intake was reduced from the day of mating to day 10 of pregnancy. A high level of feeding during rearing is associated with an increase in ovulation rate at first, second or third oestrus and with a reduction in embryo survival during early gestation (den Hartog & van Kempen, 1980; den Hartog, 1984). High energy feeding during the premating period is also connected with a reduced embryo survival during early gestation in gilts. The reason for this is unclear, but gilts having high energy intakes had also an increased ovulation rate and this may lead to an increased embryo mortality. Pharazyn et al (1991) feeding gilts with two levels of energy and protein from days 3 to 15 of gestation had no effect on plasma progesterone in early pregnancy or embryo survival to day 28. However, overall day 3 plasma progesterone concentrations were positively related to embryo survival and inversely related to variance in survival. Their hypothesis was therefore that changes in circulating progesterone in the immediate post-ovulatory period may be of greater importance in determining embryo survival than progesterone concentrations later in gestation. In trials in which embryo survival is decreased, the effect may be mediated by a reduction in plasma progesterone. Parr et al. (1993) showed results indicating that high feed levels after mating reduce peripheral progesterone concentrations and increase embryonic mortality. They also tried to correct this with exogenous progesterone and their preliminary results are promising. The mechanism underlying the reduction in plasma progesterone associated with increased feed or energy intake during early gestation is still unknown. Symonds and Prime (1988), using

ovariectomized nonpregnant gilts, showed that the portal blood flow and the metabolic clearance of progesterone from plasma (MCR) increased significantly when the food intake was increased.

Several studies have also been carried out aimed to elucidate the effect of feeding during lactation on subsequent embryo survival in sows. King & Williams (1984a,b) found no influence of low lactation dietary energy or protein intake on embryo survival. Hughes et al. (1984) found a lower embryo survival (%) in sows fed 3 kg/day compared with sows fed 7.0 kg/day despite the fact that the ovulation rate was approximately the same in the two groups of sows. In sows having excessive weight and fat losses in lactation, it is possible that an increased hepatic blood flow after weaning is increasing the metabolic clearance rate of plasma progesterone thereby reducing the plasma progesterone levels (Aherne & Kirkwood, 1985). The effects of low lactation feeding on embryo survival have also been suggested to be the result of sub-optimal LH release at the post-weaning oestrus, resulting in inadequate luteinization of the corpora lutea with concomitant low plasma progesterone levels in early pregnancy. Further support for this hypothesis was presented from a study demonstrating that injection of GnRH at the post-weaning oestrus improved embryo survival and increased progesterone levels in early gestation in sows fed low levels during lactation (Kirkwood et al., 1987). Studies on endocrine changes associated with fasting sows on day 10 and 11 of pregnancy revealed no effect on luteal function (plasma progesterone) (Tsuma et al., 1994). However, plasma concentrations of cortisol and  $\beta$ -endorphin increased and oestradiol decreased during the period of fasting. Further studies are needed in this area, in particular the link between increased concentration of cortisol, decreased concentration of oestradiol and embryo survival.

*Nutrition, metabolic and reproductive hormones and interval from weaning to oestrus*

A high feed intake in pregnancy results in a lower feed intake in lactation, due at least in part to a reduced appetite in lactation. The results from recent feeding experiments indicate that the primary objective in feeding the lactating sow is to maximize food intake. To achieve this gestation food intake must be held low, high environmental temperatures must be avoided, high density lactating sow diets should be fed, and the fat content of the sow at farrowing should be controlled (Hughes, 1989). The rate at which maternal depots are mobilized in the sow during lactation, reflected by a loss of weight and a decrease in backfat thickness, is except for lactation feeding influenced by body weight and backfat thickness at farrowing (Rojkittikhun et al., 1992), litter size and litter weight gain (Sterning et al., 1990). The variation in weight loss is also large among sows fed the same amount of feed and nursing the same number of piglets (Sterning et al., 1990). This indicates that individual differences in energy metabolism might exist between equally nourished sows with the same performance during lactation (Rojkittikhun et al., 1992). High estimate of heritability was also found for weight loss during first lactation (Rydhmer et al., 1992).

Baidoo & Aherne (1988a,b) demonstrated that plasma concentrations of FSH before weaning, and those of FSH and LH after weaning, were significantly higher in sows fed ad libitum during lactation than in comparable sows fed fifty per cent of the ad lib intake. A low level of feeding during lactation resulted in greater weight and backfat loss, higher plasma concentrations of GH and cortisol, and lower concentrations of insulin during lactation. Tokach et al. (1992) characterized LH secretion during lactation in primiparous sows that experienced an early (< 9 days) or late (> 15 days) return to oestrus post-weaning. Early sows had a higher LH mean and more LH peaks per 6 h on days 14, 21 and 28 and higher serum insulin on days 7 and 21 than did late sows (Tokach et al., 1992). They also found that insulin

concentration on day 7 was correlated with number of LH peaks on day 14, day 21 and day 28. Tokach et al. (1992) concluded that alterations in LH profile as early as day 14 of lactation are associated with a delayed return to oestrus after weaning, and that insulin levels during early lactation may be associated with subsequent reproductive function. No significant relationship between LH and insulin on either day 10 or day 20 of lactation was demonstrated by Rojkittikhun et al. (1993a). The LH secretion was also similar between the periods of low and high plasma insulin during short-term starvation and refeeding respectively (Rojkittikhun et al., 1993b). These findings indicate that the changes in plasma levels of endogenous insulin does not influence the LH release in lactating sows. Further studies are necessary to elucidate the somewhat deviating results presented by Tokach et al. (1992) and Rojkittikhun et al. (1993a,b).

Protein and energy intake during lactation influence LH secretion in an interactive manner (Tokach, 1991). At low energy intake, lysine intake had little influence on mean LH during lactation. However, the influence of lysine intake on LH secretion increased as energy intake increased, indicating that LH secretion is reduced by restrictions of either lysine or energy intake. As indicated above, different metabolites have been implicated as possible mediators between nutrient intake and reproductive function but further studies are needed.

Parts of the work reported in this paper received financial support from the Swedish Council for Forestry and Agricultural Research.

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### **Comparison of Ovarian Function and Embryo Development in Meishan and Large White Hybrid Pigs**

Hunter, M.G.

#### **Introduction**

The Chinese Meishan pig is characterised by its prolificacy, producing an average of three to four more piglets per litter than European Large-White hybrid pigs. Cross-breeding studies have indicated that this breed difference is due to genes acting in the mother, and not in the litter itself. Nevertheless, it is not yet fully resolved whether the effects occur via the oocyte, the uterus, or systemically or possibly by a combination of all three. Initial studies showed discrepancies as to whether the Meishan female has a higher ovulation rate than Large-White hybrids, but these studies were often confounded by comparisons with different animals in various reproductive states. It is now generally accepted that Meishan pigs do have a higher ovulation rate, but only after they have experienced an extended number of oestrous cycles, or parity. Although this higher ovulation rate can contribute, in part, to the prolificacy of the Meishan breed, further evidence suggests that the main cause of prolificacy in Meishan pigs is an enhanced level of prenatal survival. This advantage in prenatal survival to the Meishan pig is clearly present in the post-attachment period (after day 20 of gestation), but may also be present earlier in gestation.

#### *Endocrinology*

As part of our studies at Nottingham we have investigated both the level and pattern of circulating reproductive hormones during the periovulatory period in gilts of both breeds that had experienced 3 - 6 oestrous cycles. There were no significant differences in the mean basal concentrations of either LH or FSH in the pre-surge period, in peak surge concentrations of LH and FSH or in the duration of the LH surge, but there was a consistent trend for gonadotrophin concentrations to be higher in Meishan than in Large-White hybrid gilts. We are currently extending this study by investigating hormone secretion in older sows, in which situation the Meishan animals have a higher ovulation rate than their Large-White hybrid counterparts. The interval between peak oestradiol and the LH surge also remained constant between the breeds ( $P > 0.1$ ). However, Meishan gilts exhibited oestrus significantly earlier ( $P < 0.005$ ) than did Large-White hybrid females and this provides an endocrine basis for the extended time interval between oestrus onset and ovulation found in Meishan pigs (49 and 34h in Meishan and Large-White hybrid gilts respectively). During the periovulatory period, circulating inhibin concentrations were higher in Meishan gilts ( $P < 0.05$ ) than Large-White hybrids, although this was not accompanied by a reduction in circulating FSH concentrations. This suggests a decrease in sensitivity to the feedback of inhibin on FSH secretion in Meishan gilts and this was further supported by our finding that charcoal-stripped follicular fluid was less effective in reducing FSH in ovariectomized Meishan gilts than in ovariectomized Large-White hybrid gilts. This differential sensitivity to inhibin feedback may allow for an increased FSH:LH ratio at critical times in the oestrous cycle of Meishan pigs and may also be associated with the increased ovulation rates in older,

parous, Meishan sows.

#### *Follicular characteristics*

A comparison of preovulatory follicular characteristics between the breeds revealed some interesting differences, although, the heterogeneity, or variability, in the characteristics was similar in both breeds indicating no increase in synchrony in the Meishan breed. Nevertheless, Meishan follicles were found to be smaller and contain less follicular fluid ( $P < 0.005$ ) than their Large-White counterparts, but the follicular fluid oestradiol concentration was higher ( $P < 0.06$ ) in Meishan follicles. A further experiment showed that aromatase activity was higher ( $P < 0.05$ ) in both granulosa cells and theca tissue recovered from Meishan follicles than from Large-White hybrid pigs. This was similar during both the early and late follicular phase and provides the basis for the higher follicular fluid oestradiol concentration in the Meishan follicles, but the mechanism remains uncertain. We have also recently investigated second messenger (cyclic AMP) responses to LH by follicles from both breeds and again have found breed differences ( $P < 0.05$ ) with the Meishan follicular tissue producing higher levels of cyclic AMP.

#### *Oocyte maturation*

Since the highly oestrogenic nature of the follicles of Meishan pigs may provide a more suitable environment for oocyte maturation and subsequent fertilization, an experiment was carried out to examine follicular and oocyte maturation in ovaries recovered in the immediate preovulatory period. A total of 447 follicles was examined from the two breeds and Meishan follicles were again found to be smaller and contain less follicular fluid ( $P < 0.05$ ). Classification of oocytes from these follicles into discrete stages of meiotic development showed that oocytes of Meishan gilts were more mature than those of Large-White hybrid gilts ( $P < 0.01$ ). For 15 of the 16 Meishan gilts examined, the majority of their oocytes were in the final stages of maturation whereas for 6 of the 13 Large-White hybrid gilts the majority of their oocytes were in the less developed stages of maturation.

#### *Embryo development and the uterine environment*

To determine whether the later LH surge relative to oestrus onset is important for the high rate of survival in the Meishan pig we advanced the LH surge by administering hCG to Meishan gilts at the onset of oestrus. While ovulation rate was unaffected compared to control untreated animals, the number of live conceptuses and embryonic survival rate on Day 30 of pregnancy were reduced ( $P < 0.1$ ) by hCG treatment. In addition, mean placental weight was increased ( $P < 0.01$ ) and foetal weight and placental length tended to be increased ( $P < 0.07$ ) in conceptuses recovered from hCG treated gilts. Serum progesterone concentration did not differ with treatment overall but was significantly elevated ( $P < 0.05$ ) at 48h post-oestrus in the hCG treated gilts which was consistent with earlier ovulation in these animals. A further study was designed to study the involvement of the intrauterine steroid environment in embryonic survival in the Meishan pig, by comparing conceptuses recovered on Day 30 of pregnancy from Meishan and Large-White hybrid animals. The results showed that although embryonic survival was higher in Meishan animals, foetal weights, placental lengths and allantoic fluid volumes were all lower ( $P < 0.01$ ) than in their Large-White counterparts. Furthermore, placental oestradiol synthesis *in vitro* by tissue recovered from Meishan animals was significantly ( $P < 0.001$ ) reduced compared to placental tissue recovered from Large-White hybrid animals. This indicates a difference in the intrauterine steroid environment between the two breeds. Indeed studies by others suggest the presence of factor(s) in endometrial secretions from Meishan females that reduce the growth rate and oestrogen secretory potential of preimplantation conceptuses.

We have shown that the Meishan preovulatory follicular population is more oestrogenic and shows a different pattern of second messenger responses compared with European breeds. Furthermore, ovulation occurs later in oestrus and the oocyte population is more mature prior to ovulation, which would ensure rapid, synchronous fertilization and embryo development. Advancement of the LH surge to coincide with the onset of oestrus had a detrimental effect on embryo survival by Day 30 of pregnancy. The intrauterine environment also contributes to the prolificacy of the Meishan pig, by restricting conceptus growth and oestrogen secretion by the placenta.

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### **Recent Acquisitions in Pig Oocyte Maturation and Fertilization In Vitro**

Mattioli, M.

In recent years an increasing knowledge on the physiology of gamete growth and maturation has accumulated, thus making it possible to reproduce in vitro a complete series of events, from oocyte maturation and sperm capacitation to fertilization and early embryo development. Although the results obtained are encouraging, further research is required in order to increase the efficiency of both oocyte maturation and fertilization in vitro.

#### *Oocyte maturation*

Oocyte maturation in vivo occurs in the presence of highly concentrated somatic follicle cells. In vitro, exhaustion of medium and accumulation of catabolites and free radicals impose a limit to the number of cells that can be used and this is particularly stringent for pig oocytes that have a maturation time of 44 h which is nearly twice as long as in ruminants or three times longer than in rodents. Cell supplementation of the culture medium has, however, been carried out resulting in an improved developmental competence of IVM oocytes (Mattioli et al., 1988, Ding & Foxcroft, 1992). Similar positive results were obtained by using follicular fluid (Naito & Fukuday Toyoda, 1988). Experimental evidence has been provided that the positive effect of somatic cells is at least in part attributable to their positive influence on the cumulus-oocyte heterologous coupling; this is maintained for most parts of the maturation only if cumulus-oocyte complexes are cultured in the presence of abundant somatic tissue (Mattioli et al., 1988). A major advantage resulting from this persistent interaction between cumulus cells and oocyte is the acquisition of the factors in the ooplasm, required to decondense penetrated sperm and form male pronuclei. Inability to perform this function has often been the major abnormality of pig oocytes matured in vitro. This abnormality seems to depend on an inadequate reducing activity of the ooplasm (Zirkin et al., 1985). The persistent cumulus oocyte interaction throughout maturation seems to be required to support sperm decondensation. A completely different approach has recently been used to overcome this problem (Nagai, 1994). After realizing that more reducing agents were required in the ooplasm, the medium was supplemented with cystein as a precursor of intracellular GTH. Under this condition oocytes forming male pronuclei were obtained without any coculture. Experiments of oocyte electrical activation suggest a further deficit contributing to this abnormality. In vitro matured oocytes may contain low levels of active MPF, insufficient to break the nuclear membrane of penetrated sperm and preventing the sperm decondensation and male pronucleus formation (Funahashi et al., 1993).

The use of gonadotropins in in vitro maturation systems has been discussed controversially. Naturally, oocyte maturation occurs only in preovulatory follicles exposed to peak levels of gonadotropins, while in vitro the oocytes mature in the presence of gonadotropin hormones almost regardless to the size of the follicle from which they originate. Generally, when an abundant somatic component is used, gonadotropins increase the developmental competence of IVM oocytes. Brackett et al. (Brackett et al., 1989) clearly showed that very high levels of

LH selectively improve the developmental competence of IVM in bovine oocytes and we have demonstrated that LH but not FSH improves the fertilizability of IVM pig oocytes (Mattioli et al., 1991). A transient increase in intracellular cumulus-oocyte coupling could mediate this effect. Nevertheless, fully competent oocytes have been obtained in many species without gonadotropins in the culture medium. The effect of LH on the oocyte is still largely unknown. After the ovulatory surge, LH reaches the follicles, reverses the steroidogenesis of the follicle and then it is cleared off by blood circulation. Funahashi and Day (Funahashi & Day, 1993) mimicking this event, have improved the quality of IVM oocytes by replacing hCG-PMSG supplemented medium with hormone free medium after one day of culture.

In vitro and in vivo studies have shown that LH causes a depolarization of the membrane potential of somatic cells and consequently of the electrically coupled oocyte (Mattioli et al., 1991). Electrical impulses are able to open  $Ca^{++}$  channels present in the oolemma and cause an intracellular accumulation of  $Ca^{++}$  ions. Higher  $Ca^{++}$  levels have been shown to accompany oocyte maturation. In the pig the mechanism of this regulation is peculiar and different from that of rodents. LH induces somatic follicle cells, particularly granulosa cells from large follicles, to release a second messenger which acts directly on the oocyte, presumably on the oolemma and stimulates adenylate cyclase. In all oocytes maturing in vivo, LH induces an increase in cAMP levels at the beginning of maturation that, as it has been shown under in vitro conditions, accelerates meiotic progression (Mattioli, 1992). Such an increase can be obtained in vitro only if cumulus oocyte complexes are cultured with follicle shells or granulosa cell suspensions stimulated with LH. However, live piglets have been obtained from cumulus oocyte complexes matured alone, without any tissue or cell supplementation and therefore without any cAMP raise.

All these data combined suggest that fully grown oocytes have their own maturation program. In vivo it is finally tuned by the follicular environment and a correct execution is guaranteed. The attempt to use similar regulatory compartments for oocyte maturation in vitro has helped to avoid some abnormalities in the development. However, the finding that even oocytes that did not experience all the events characterizing maturation in vivo showed good developmental competence under in vitro conditions, suggests that mimicking the natural process may not always be necessary. In vitro we are operating on a much simpler model and therefore many prerequisites occurring naturally may be unnecessary. However, different kinds of control mechanism, like growth factors, neuromediators, antioxidants etc., may be of advantage.

#### *In vitro fertilization*

Despite the wide intensive research on IVF techniques, the knowledge of the functional status of the spermatozoa that are introduced in the fertilization system is still scarce. After incubation to induce capacitation within the same sperm sample a wide range of uncapacitated, capacitated and acrosome reacted spermatozoa as well as variable amounts of immotile and dead sperm are present. Such individual status may even change through sperm-oocyte incubation.

The limited knowledge of these aspects are in part due to the fact that capacitation is generally considered as a distinct event before fertilization but its completion has been evaluated only by tests using in vitro fertilization. The absence of a precise knowledge on the functional status of the sperm population introduced in the IVF system makes it difficult to establish the correct sperm concentration, therefore a large number of spermatozoa are generally used in order to guarantee satisfactory fertilization rates. The heterogenous composition of the sperm sample and the longlasting sperm-oocyte co-incubation create a dynamic condition where the

oocyte has to face subsequent waves of spermatozoa that complete capacitation and then initiate their attempt to fertilize. This frequently results in a polyspermic fertilization, a major problem in pig IVF. New approaches are available to obtain information on the sperm status. The identification of capacitated sperm can be achieved by monitoring intracellular free calcium in spermatozoa exposed to solubilized zonae pellucidae (Personal observations). Calcium transience is the expression of a reactive condition typical for capacitated spermatozoa. The analysis of the acrosome integrity with specific fluorescent stains upon exposure to zonae pellucidae can also be used assuming that only capacitated sperm undergo zona-induced acrosome reaction (Cross et al., 1986). Finally, the fluorescent pattern of spermatozoa stained with CTC can be used to routinely assess the proportion of capacitated boar sperm (Mattioli et al., 1993). These approaches can be conveniently used to identify the culture conditions that are most suitable to obtain a rapid and diffuse sperm capacitation and evaluate the potential for the use of specific stimulators. With these investigations progesterone was shown to specifically promote and accelerate the capacitation process in boar sperm, thus improving its fertilizing ability in vitro (Mattioli et al., 1993). A reliable and rapid diagnosis of capacitation could help to perform a technique for the selection of capacitated spermatozoa. With such a preparation polyspermy could probably be prevented by using low sperm concentration and short incubation intervals. At present, in the absence of methods for the selection of spermatozoa, the attempts to reduce polyspermy are generally based on the use of modulators of the fertilizing ability of the spermatozoa. Encouraging results were obtained using oviductal cell monolayers (Nagai et al., 1990). Molecules derived from the oviduct can reduce polyspermy by acting directly on the sperm rather than the oocyte. Recent studies by Funahashi and Day (1993) indicate that the addition of porcine follicular fluid can also be used to reduce polypermic fertilization.

Furthermore, BSA may be used with the same purpose. Since BSA may inhibit meiotic progression its addition during the second half of oocyte maturation in vitro is sufficient to create the conditions for a significant reduction of polyspermic fertilization (Zheng & Sirard, 1992).

The effect of ionic composition of the medium on the rate of polyspermy has been investigated and high calcium is frequently used in the medium to allow the dispersal of the cortical granules content in the perivitelline space necessary for an efficient block to polyspermy (Cran & Cheng, 1985). Unfortunately, high calcium concentration favourably affects the fertilizing ability of boar spermatozoa, thus causing a parallel increase of polyspermy.

The recent acquisitions presented have further improved the efficiency of IVM-IVF of pig oocytes. The rapidly growing knowledge of the molecular mechanism governing oocyte maturation will probably allow to further improve these techniques with strategies directly focussed on the series of specific check points that the oocyte has to overcome to mature and acquire full developmental competence.

References are available from the author.

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### **Gamete Intrafallopian Transfer (GIFT), an alternative to in vitro Fertilization Procedures for Special Applications**

Rath, D., Niemann, H. and Johnson<sup>1</sup>, L.A.

Protocols for porcine in vitro fertilization (IVF) still hamper a routine application because of inadequate maturation, fertilization and culture conditions although some improvements have been described recently.

In human similar difficulties exist and were partly overcome by gamete intra fallopian transfer (GIFT). The procedure has been developed as an alternative to IVF and embryo transfer (ET). A reliable GIFT technology for livestock species would offer significant advantages in particular with gender sorted semen. The objective of the present experiments was to develop a GIFT system for the pig to learn more about the developmental capacity of the embryos produced. The technology should form the basis for the use of sex separated semen and the study of the development of the resulting embryos.

Therefore a specific transfer catheter was developed to allow aseptic transfer conditions and prevent further microscopic handling of the gametes at surgery. Oocytes and spermatozoa were placed into french straw (250 $\mu$ l). To prevent damage of the oviductal epithel cells through the transfer procedure the tip of the straws was smoothed by a 5 mm Teflon tube.

In Experiment 1 (6 replicates) 234 oocytes ( $39 \pm 5.5$  per recipient) plus spermatozoa (4000 to 8000 per oocyte) were transferred bilaterally into the oviducts of synchronized gilts, and embryos were recovered 48 h thereafter. The recovery rate was 50.4% and 50% of the recovered oocytes were fertilized. A total of 55 embryos was cultured in vitro in NCSU-medium for 48 h and 63.6% developed to morula or blastocyst stages.

In Experiment 2 (5 replicates) 220 oocytes ( $44 \pm 4.9$  per recipient) plus spermatozoa (4000 per oocyte) were transferred to 5 recipients which were allowed to go to term. Three gilts delivered 16 (n = 3, 5, 8) piglets.

In Experiment 3 (5 replicates) 183 oocytes ( $36.6 \pm 1.2$  per recipient) plus flow cytometry gender sorted spermatozoa (4000 per oocyte) were transferred to 5 recipients. The recovery rate was 47.8%, and 27.6% of the oocytes were fertilized. From all cleaved oocytes 45.8% developed to expanded blastocysts, with the number of blastomeres varying from 20 to 85 ( $38.3 \pm 22.5$ ).

The results of our study indicate that the GIFT procedure can be used successfully in the pig and yield normal offspring. Up to now this technology has only been used in human and it was shown that GIFT was superior to in vitro fertilization (Society for Assisted Reproduction Technology, 1988). Our results also suggest that GIFT may be superior to IVF for use in the pig (Rath et al. 1994).

Today, success rates following porcine in vitro fertilization are still low and do not exceed a 40% fertilization rate. Pregnancies have been established only occasionally (e.g. Yoshida

1987; Mattioli et al. 1989; Rath 1992). The pregnancy rate obtained in this study compares favourably with that in human in which pregnancy rates of 40% were achieved (Barad et al. 1988; Herwitt, 1991). Although piglets have been obtained and regular full-term development has been completed from GIFT-derived embryos, the procedure needs further improvements to allow for a broader application of the technology for specific experimental purposes. Based on experiences from IVF, approximately 4000 qualitatively non selected spermatozoa per oocyte had been transferred in our experiment. This is still far from normal conditions in the oviduct and future experiments have to determine the optimal number of spermatozoa that in our present study were related to the number of transferred oocytes.

Similarly, the total volume of the medium (~ 80 µl) may affect gamete interaction and early embryonic development occurring in the oviducts. Physiologically, the secretion of oviductal fluid during estrus varies from 0.8 ml (rabbit) to 4.1ml (cow) per 24 h (Hunter 1980), and oviductal cells in sows produce approximately 6.3 ml/24 h (~4.4 µl/min; Iritani et al. 1974). Thus, we injected the total volume within 15 sec that is equivalent to physiological secretion over approximately 20 min.

A significant effect of the number of transferred oocytes on the pregnancy rate has been shown in human. No pregnancies were obtained following transfer of only 1 oocyte, while the pregnancy rate increased to 43% after transferring 4 oocytes per recipient. But one has to bear in mind that the pig, as a polytocous species, might differ substantially from the human in terms of the physiological conditions in the fallopian tubes. Furthermore, in humans the site of gamete deposition has a major effect on the pregnancy rate.

The donors were asynchronous with the recipients by 4 h, which ensured that the latter had not yet ovulated at the time of transfer. It may be beneficial to increase this time interval to better adapt the estrous cycle of the recipients to the developmental delay of the oocytes that normally occurs in the in vitro procedure. Finally, it has to be established whether ectopic pregnancies occur as observed in humans (Li et al. 1992).

The results of Experiment 3 show that viable embryos can be obtained from GIFT with sperm sorted for X and Y chromosomes. Fertilization rates were reduced compared with those in Experiment 1 using nonsorted spermatozoa; but the rates were better than after in vitro fertilization with gender sorted semen. As indicated by a reduced sperm motility the lower rate is mainly attributed to the increased sensitivity of the spermatozoa after flow cytometric treatment.

This experiments clearly show that offspring can be produced by the GIFT system and under specific conditions it is an alternative method to IVF.

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### **Nuclear Transplantation in Mammals: The Role of Maturation Promoting Factor (MPF)**

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At present, two nuclear transplantation procedures (cloning) are commonly used:

- I. Metaphase II enucleated oocytes (cytoplasts) are fused to early embryonic cells,
- II. Metaphase II cytoplasts are activated 4 - 6 h before fusion to early embryonic cells.

The transplanted nuclei are thereafter exposed to two different environments. It is generally accepted that in the first case, these nuclei are exposed for a short time to MPF, which is present in M II cytoplasts but disappear rapidly after activation (Fulka, Jr. et al., 1992). MPF thereafter induces nuclear membrane breakdown and premature chromosome condensation (PCC). However, the drop in MPF activity results later on in decondensation and formation of (pro)nuclei-like structures with a different degree of remodelling (Kanka et al., 1991). In the second case, no MPF is present in the cytoplast; as a logical consequence, the transplanted nuclei remain unchanged. While in the mouse the development is much better when transplanted, nuclei are exposed to MPF (Cheong et al., 1993), quite different results were published in cattle and sheep. Here, the second approach seems to be much more promising (Loi et al., 1993).

Recently we have developed the non-invasive chemical procedure of mouse oocytes (Fulka, Jr. and Moor, 1993). Using this technique, we are able to produce a large quantity of cytoplasts with high levels of MPF. This enables us to study the role of MPF upon remodelling of transplanted nuclei and some factors which may influence the success of nuclear transplantation under those conditions. Some of the results will be reported here.

Under our standard procedure, the embryonic cells are fused to enucleated oocytes and activated 90 min later. The result of activation are two cells of the same size; each containing a perfect nucleus. Each nucleus contains a dense, round shaped nucleolus (I). It means that under these conditions we are able to return a very advanced nucleoli (even from stem cells) back to the earliest morphological stage which is typical for pronuclear nucleoli. The interval between fusion and activation (90 min) seems to be very important. In about 30 min post fusion nuclear membrane breakdown and chromosome condensation are observed; however, the chromatin is still disorganised. Metaphase plate and spindle formation are improving with time, and under our conditions this is finished 90 min post induction of fusion. The morphology of M-plates depends on the blastomere cell cycle stage. S-phase nuclei form these M-plates with some joined and some fragmented chromatin (spindle is always present), but we can hardly believe that after activation the separation of chromosomes is equal. On the other hand G2 nuclei form perfect M-plates and the separation of chromatids after activation seems to be normal. Our recommendation

thereafter is: If transplanted nuclei are to be exposed to MPF, activate the cytoplasm-embryonic fusion products at about 90 min post fusion. It is absolutely necessary to use nuclei which are in G2 stage of the cell cycle (Fulka, Jr. et al., 1993).

However, in large domestic animals the chemical enucleation procedure does not work very well. For this reason it is necessary to enucleate these oocytes mechanically by removing the M II-plate using an enucleation pipette. The early embryonic cells are fused to cytoplasts; however the question is: are the transplanted nuclei always exposed to MPF? Our preliminary results clearly showed that this is not always true (Ouhibi, Fulka, Jr. and Moor, in preparation). For this reason we analyzed the levels of MPF in bisected mouse oocytes. Briefly: the oocytes (shortly after GVBD, MI, MII) were bisected into two equal halves which were thereafter fused to S or G2 phase 4-cell blastomeres. While in nucleated halves, PCC in transplanted nuclei was always induced irrespective of the cell cycle blastomere stage; in enucleated halves only G2 nuclei responded to the cytoplasmic signal. S-phase nucleus breakdown was induced only in cytoplasts produced shortly after GVBD, while in MI and MII enucleated halves the transplanted nuclei remained intact. The gradient of MPF is in close correlation with spindle formation. The half with spindle always contains the levels of MPF which are much higher than the levels in the sister half. This hypothesis is supported by our next experiment when spindles were destroyed by preincubation in colcemide. In this case both halves induced PCC irrespective of the cell cycle blastomere stage. Thus it is clear that MPF is somehow bound to the spindle (Kubiak et al., 1993).

In conclusion our results clearly show:

1. MPF has a beneficial effect upon the remodelling of transplanted nuclei
2. The exposure time to MPF must be approximately 90 min
3. Only G2 nuclei must be used for fusion
4. When cytoplasts are produced by mechanical enucleation, incubate the oocytes in colcemide before MII chromosomes are removed. Thus, you will increase the levels of MPF in cytoplasts.

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### Counter Current Transfer of Hormones in Pig

Krzymowski, T., Stefanczyk-Krzymowska, S., Grzegorzewski, W. and Skipor, J.

The commonly accepted opinion on the hormonal mechanism for the estrous cycle regulation is based on the fact that uterus, oviduct, ovaries and vagina, as well as the central nervous system and pituitary gland are influenced by changes of hormone concentration in the systemic arterial blood system supplying these organs, as well as receptors concentration in target organs. Many results from the last decade regarding the local mechanisms for hormone concentrations allow us to revise, enlarge and modify this hypothesis.

#### *I. Local influence of the ovaries on the uterine function*

In 1976 the preliminary results were demonstrated regarding the counter current transfer of progesterone from venous effluent to arterial blood supplying the ovaries in sheep (McCracken & Einer-Jensen, 1976). Intensive studies were performed with sows. Tritiated testosterone, estradiol and progesterone introduced into the ovarian vein were found in high concentrations in the convoluted ovarian artery branches (Krzymowski et al., 1982a, 1982b). Morphological adaptation of periovarian vascularization to counter current exchange of hormones was demonstrated (Doboszynska et al., 1991, Krzymowski et al., 1990). The vessels taking up blood from the ovary form a dense network which entwines small arterial vessels carrying blood to the ovary and forms a vein-vein network over an extended area of small looping branches of the ovarian artery (Doboszynska et al., 1991). In contrast, the very thin walls of lymphatic vessels near the ovary are covered by a mesh of arterial vessels supplying blood to the ovary (Doboszynska et al., 1991, Krzymowski et al., 1990). It has been suggested that counter current transfer of steroid hormone provides for a local mechanism of action by the theca and granulosa cells for regulation of their activity through a local feedback mechanism (Krzymowski et al., 1989, 1990).

However, in 1983 it was demonstrated in sows that blood from ovarian artery branches directly supplying the oviduct does contain a 5-10 fold higher concentration of steroid hormones compared to systemic blood (Hunter et al., 1983). It was also demonstrated that plasma progesterone concentrations in the veins draining the oviduct were significantly greater than in the jugular or uterine veins during early pregnancy in the pig (Pharazyn et al., 1991).

Recently, a new experimental model has been used in our laboratory (Stefanczyk-Krzymowska et al., 1990). On both sides of the uterus, catheters were inserted into branches of the uterine artery proximal to the ovary. The tip of each catheter was placed close to, but above anastomoses with ovarian artery branches. This enabled collection of uterine arterial blood mixed with ovarian arterial blood supplying the uterus and oviduct without any damage to the ovarian vessels. A third catheter was placed in the jugular vein. Simultaneous blood samples were collected from anaesthetized animals during surgery and when the surgery was

completed, as well as from conscious animals on Day 2 after surgery. Blood plasma samples were assayed for progesterone and androstenedione (on Day 10 of the estrous cycle) and androstenedione and estrone (on Day 18 of the estrous cycle).

In all experiments it has been demonstrated that plasma concentrations of steroids was greater in the branch of uterine artery than in the jugular vein as follows: - progesterone -35%, androstenedione -46%, - during the luteal phase, (Stefancyk-Krzymowska et al., 1994, in press) and estrone -66% and androstenedione -36% - during the follicular phase (Stefancyk-Krzymowska, unpublished).

We concluded that elevation of steroid hormone concentrations in the investigated branch had to be a result of hormone concentration due to the counter current mechanism in the ovarian vasculature. We suggest that these local rises of androstenedione and progesterone concentrations have to influence anabolic processes in uterine tissues during the luteal phase. Increases of progesterone and estradiol concentrations could stimulate vasoconstriction (progesterone) and vasodilatation (catecholestrogens) of the uterine artery and its branches. No research has been done sofar to study the concentrations of oxytocin and relaxin produced in the ovary, although their counter current transfer to the ovarian artery in sheep (Schramm et al., 1986a, 1986b) and pigs (Koziorowski et al., 1989) was demonstrated earlier.

### *II. Local influence of the uterus on ovarian function*

The Bazer and Thatcher theory, first published in 1977 and presented later by the authors and their collaborators in many papers, is based on the assumption that  $\text{PGF}_{2\alpha}$  is secreted in the endocrine direction toward the uterine vasculature in cycling gilts, and it is thus transported to the corpus luteum to exert its luteolytic effect. But in pregnant and pseudopregnant pigs, the direction of secretion for  $\text{PGF}_{2\alpha}$  is exocrine into the uterine lumen.

In the last decade the majority of published papers on the exocrine versus endocrine mechanism were based on a method wherein bilateral perfusion devices were utilised for measurement of prostaglandin secretion by luminal and myometrial surfaces of porcine endometrium (Gross et al., 1988, Lacroix & Cann, 1983). According to this method endometrium was dissected from myometrial tissue and each sample was placed into a bilateral perfusion device which allowed separation of myometrial and luminal surfaces. We have a very critical opinion of this model and to the Bazer and Thatcher theory. In our opinion, a small sample of separated endometrium- deprived blood and lymph circulation, incorporating large damage and with many uterine glands open on the myometrium surface, can not be a suitable or precise model for the study of exocrine and endocrine hormone transfer. In our opinion, the Bazer-Thatcher theory is until now only hypothesis and should still be considered exactly as such.

Since 1986 we have presented many results demonstrating a new, unknown mechanism for prevention of luteal regression during pseudopregnancy and pregnancy. When  $^3\text{H-PGF}_{2\alpha}$  was infused in many sites into the most superficial layer of the mesometrium, just near the myometrium (Krzymowski et al., 1986, 1987) or when it was injected into the lumen of the uterine horn (Krzymowski et al. 1986, 1987, Stefancyk-Krzymowska et al., 1990), labelled prostaglandin was found in the arterial blood plasma taken from the uterine artery branches and in the uterine flushing. We concluded that the ability of the mesometrial vasculature to take up  $\text{PGF}_{2\alpha}$  and the intense back transfer through the arterial vessels of the broad ligament into the uterus is involved in the mechanism of corpus luteum protection against luteolysis and this way may play a very important role in the maintenance of corpus luteum functioning during early pregnancy and pseudopregnancy.

On the basis of all our results and the results of others, we suggest the existence of the following physiological mechanism:

1. Influence of catecholestrogens on alpha-adrenergic receptors is strongly decreased after ovulation. Increase of systemic and local progesterone and androgen concentrations stimulates anabolic processes in uterine tissues as well as elevating the progesterone: estradiol ratio which constricts the arterial vessels. Progesterone is highly correlated with the density of alpha-adrenergic receptors and estrogen is negatively correlated with the noradrenaline available for activation of that receptor (Ford et al., 1979, Ford et al., 1985, Reynolds & Ford, 1984). As a result, blood flow through the mesometrial and uterine vessels (Ford & Christenson, 1979) as well as  $\text{PGF}_2\alpha$  retrograde transfer (Krzymowski et al., 1986) are gradually reduced during the late luteal phase.
2. If the concentration of  $\text{PGF}_2\alpha$  rises in the venous and lymph effluent during the luteal phase,  $\text{PGF}_2\alpha$  is retrograde transferred to the uterus with arterial blood according to a counter current mechanism and thus CL is protected against luteolysis (Krzymowski et al., 1986, 1989, 1990).
3. Uterine blood flow during the estrous cycle falls to a minimum during the late luteal phase (Ford & Christenson, 1979). When demand for oxygen and energy in the uterine tissue is much more than the oxygen supply (Days 13,14,15) and efficiency of  $\text{PGF}_2\alpha$  counter current transfer is reduced, (Krzymowski et al., 1986,1989, 1990) the epithelial cells of the uterine gland and uterine lumen reduce their activity and violently release their product ( $\text{PGF}_2\alpha$ ) into the nearest environment. The majority of  $\text{PGF}_2\alpha$  flows with lymph and venous blood to the lymphatic plexus placed near the ovary (Gawronska, unpublished) as well as to the utero-ovarian vein (Heap et al., 1985), and it is passed by the counter current mechanism into CL. However, on Day 14-18 during pseudopregnancy after earlier estrogens injection (Krzymowski, 1987) and on Day 15-18 of pregnancy (Stefanczyk-Krzymowska, 1990) (embryo estrogens, PGE), the very active retrograde transfer of  $\text{PGF}_2\alpha$  to the uterus was demonstrated. A large portion of prostaglandins was back transferred from venous and lymphatic effluent to the uterus with arterial blood and was present in the uterine flushing (Krzymowski, 1987, Stefanczyk-Krzymowska, unpubl.). This indicated that it is not the exocrine versus endocrine mechanism, but the hormonal and neural regulation of blood flowing through the uterine artery and its branches, and the counter current retrograde transfer of  $\text{PGF}_2\alpha$  in the broad ligament vasculature, that play an important role in corpus luteum protection against luteolysis.

### *III. Retrograde counter current transfer of reproductive hormones in perihypophyseal vasculature*

Recently, a new area has been discovered (Krzymowski et al., 1992) where reproductive hormones were exchanged through a counter current mechanism. It was demonstrated that neuropeptides LH-RH and beta endorphin (Krzymowski et al., 1992) as well as oxytocin (Grzegorzewski, in press), progesterone (Krzymowski et al., 1992) and testosterone (Grzegorzewski, unpubl.) were transferred from venous blood into the perihypophyseal cavernous sinus to arterial blood supplying pituitary gland and brain. This transfer depended on the reproductive state of animals.

The large part of venous effluent from the pituitary gland the brain, and from the olfactory system flows through the perihypophyseal cavernous sinus into the jugular vein. The

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The large part of venous effluent from the pituitary gland the brain, and from the olfactory system flows through the perihypophyseal cavernous sinus into the jugular vein. The

cavernous sinus lies intracranially on both sides of the pituitary gland and under the dura mater. The carotid rete mirabile is located on the route of the venous blood stream and inside of this sinus. Arterial blood supplying the brain and hypophysis flows through the carotid rete and through the Circle of Willis to the brain. The approximation of venous and arterial blood in this area seems to be much closer than found in the ovarian vasculature. Experiments were carried out using an isolated head supplied during 30 min through the right carotid artery with autologous, oxygenated and heated blood immediately after exsanguination.  $^{125}\text{I}$  labelled hormones were infused into the cavernous sinus for 5 min through the angularis oculi vein (the facial vein was ligatured). Radioactive hormones were found (or not) in the arterial blood supplying the hypophysis and brain. The blood was taken from the carotid rete, through the carotid artery on the opposite side to the blood supply.

A new experiments in sows demonstrated that LH-RH and oxytocin are retrograde transferred from the cavernous sinus to the pituitary gland and the brain very strictly during 2 days after the onset of ovulation and during late luteal phase (Day 12-14) of the estrous cycle. From Day 14 after ovulation and from Day 3 to Day 12 no transfer has been demonstrated. In ovariectomized gilts estradiol benzoate or corn oil (control) was injected. Forty hours after estradiol injection (negative feedback for LH surge) LH-RH retrograde transfer from venous to arterial blood in the cavernous sinus - carotid rete complex was demonstrated. During positive feedback for LH-surge (60 and 70 h after estradiol injection) or after corn oil injection (control) no LH-RH retrograde transfer existed.

In conclusion we suggest that the ovaries, oviduct, and uterus as well as the hypophysis and brain are supplied by arterial blood with the hormone concentration changed by a local mechanism based on the permeability of vascular vessels and passive and/or active hormone transfer and exchange. In our opinion, this has an important and until now unappreciated consequences for the general regulation of female reproduction.

Literature available from the authors.

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### **The Influence of Longtime Active GnRH-Agonist on Hormonal Level and Follicular Development in Cyclic Gilts**

Brüssow, K.-P.<sup>1</sup>, Schneider, F.<sup>1</sup> and Ratky, J.<sup>2</sup>

#### **Introduction**

Gonadotropin-releasing hormone (GnRH) secreted from the hypothalamus in a pulsatile manner stimulates both the synthesis and the release of LH and FSH from the pituitary (Knobil, 1985). The gonadotropins are responsible for follicular growth and development, ovulation and maintenance of the corpus luteum.

Application of appropriate doses of exogenous gonadotropins to prepubertal and synchronized cyclic gilts, weaned and anestrus sows results in follicular growth and ovulation (Casida, 1935; Hunter, 1972; Schilling & Cerne, 1972).

GnRH induces the release of LH and FSH when given intravenously, subcutaneously or intramuscularly to pigs. Synthetic GnRH stimulates ovulation in gilts and sows following estrus synchronization (Brüssow & Bergfeld, 1979; Bergfeld et al., 1987). Follicular development and fertile estrus can be induced by pulsatile administration of GnRH in lactating sows (Britt et al., 1985), weaned anestrus sows (Armstrong & Britt, 1985) and prepuberal gilts (Lutz et al., 1985).

A long term treatment of GnRH, especially of GnRH agonists, results in the paradoxical effect of reversible inhibition of gonadotropin release, synthesis and secretion of gonadal steroids (Knobil, 1985; Rommerts & Themmen, 1986; Schneider et al. 1992a; Brüssow et al., 1993). The precise mechanism by which GnRH provokes desensitization (i.e. decreased ability of GnRH to stimulate LH release from the gonadotrope) remains unclear (Hawes et al., 1992).

The objectives of the present studies were to evaluate the influence of GnRH agonist with controlled release depot formulation on the LH- and steroid levels, follicular development and on ovarian and uterine LH- and steroid receptors in cyclic gilts.

#### **Materials and Methods**

##### **Experiment 1**

This experiment was conducted to determine the effect of GnRH agonist (GnRH-A) with controlled release depot formulation for 28 days on the LH- and steroid levels and on ovarian development in preovulatory and midcyclic gilts.

Fifteen cyclic crossbred gilts exhibited two normal estrous cycles were injected intramuscularly (d 0) with 7.5 mg triptorelin (D-Trp<sup>6</sup>-LHRH, Decapeptyl®, FERRING Kiel), micro-encapsulated in biodegradable material, on day 18 (group 1, n=5) or day 10 (group 2, n=10) of the estrous cycle. Blood samples were collected via intravenous catheters once daily

(08:00 h) and frequently (0.5-2 h interval) on d 0, d 14 and d 28 after GnRH-A. A LHRH bolus (0.3 mg Lutrelef<sup>®</sup>) was injected on d 14 and d 28 to test the receptivity of the pituitary. Ovarian development was adspected on d -2, d 12 and d 21 by means of laparoscopy (Brüssow et al., 1990a) and after slaughter on d 32.

#### Experiment 2

The influence of Decapeptyl<sup>®</sup> with controlled release of 28 days on pulsatile LH release was evaluated in this experiment.

Four cyclic gilts were injected on day 10 of estrous cycle (d 0) with 7.5 mg GnRH-A (D-Trp<sup>6</sup>-LHRH, Decapeptyl<sup>®</sup>). Four gilts injected with vehicle at the same day of oestrous cycle served as control. Blood samples were collected via intravenous catheters once daily (08:00 h) and frequently every 20 min for 6 h (08:00-14:00 h) on d 2, d 4, d 6, d 8, d 10, d 12 and d 14 following GnRH-A or on days 12, 14, 16, 18 and 20 of estrous cycle in control gilts, respectively. Ovarian development was adspected by laparoscopy on d -2 and at slaughter 2 days after completion of sampling period.

#### Experiment 3

This experiment was conducted to determine the dynamics of LH and steroid receptor contents in ovarian and uterine tissues on different days after GnRH-A administration. Thirty cyclic gilts were randomly assigned to 2 groups. On day 10 of estrous cycle (d 0) they were injected with 7.5 mg Decapeptyl<sup>®</sup> (group UNIDECA) or vehicle (group UNICONT). Three gilts of each group were unilaterally ovariectomized (left side) by means of electrocauterization on d 0, d 5, d 10, d 15 and d 20 after GnRH-A. The contralateral side was ovariectomized 10 days later, respectively. Additionally, 12 cyclic gilts (group CONT) were bilaterally ovariectomized on days 5, 10, 15 and 20 of the estrous cycle.

#### Hormone and receptor analysis

Luteinizing hormone, estradiol-17 $\beta$  and progesterone were estimated with validated RIA methods. Receptors for estrogens and progesterone were analyzed according to Lampe & Schneider (1990). LH/hCG receptors were determined using a modified method of Ziecik et al. (1986).

### Results

#### Experiment 1

Irrespective of the day of estrous cycle there was an initial rise in plasma LH after GnRH agonist application. The duration of LH peak was observed for 4-8 h with mean maximum levels of  $9.3 \pm 2.9$  (group 1) and  $10.5 \pm 5.0$  ng/ml (group 2). After the initial increase LH serum concentrations remained at basal level and were not affected by LHRH bolus stimulation. The administration of GnRH-A induced ovulation in preovulatory gilts. Serum concentrations of progesterone gradually decreased after luteolysis both in preovulatory and midcyclic gilts and remained, under the influence of GnRH-A, at basal level up to the end of the experiment. The mean estradiol-17 $\beta$  concentrations were at basal level (20 pg/ml). There were no estradiol concentrations > 40 pg/ml lasting several days and indicating follicular development. According to repeated laparoscopic ovarian observation there was a nearly complete suppression of ovarian follicular development. The ovarian size was reduced by 50 %.

#### Experiment 2

The frequency of LH pulses was different between cyclic and GnRH-A treated gilts. LH pulses were abolished after GnRH-A. In cyclic gilts there was an increase in pulse frequency from  $0.5 \pm 0.5$  pulses/6 h on d 12 to  $3.0 \pm 1.0 - 1.7 \pm 0.5$  pulses on d 16-20. But the pulse amplitude significantly decreased in cyclic gilts on d 18-20 compared to d 12. The basal LH level was not affected by GnRH treatment but increased according to the estrous cycle in cyclic gilts. The mean maximum concentration of LH pulses decreased from  $2.22 \pm 0.28$  ng/ml on d 12 to  $0.95 \pm 0.09$  ng/ml on d 20 in cyclic gilts. The LH concentration of only three estimated pulses in GnRH-A treated gilts on d 16-20 was between 0.94 and 1.52 ng/ml, respectively.

#### Experiment 3

Cyclic changes were estimated in LH/hCG-, estrogen and progesterone receptor levels. In control gilts (CONT, UNICONT) LH/hCG receptor level in corpora lutea increased after ovulation and the highest amount was determined during the early luteal phase of estrous cycle. From day 10 to day 15 and to day 20 of cycle (d 0, d 5 and d 10 after GnRH-A, respectively) the receptor level decreased and was extremely low after luteolysis. In GnRH-A treated gilts (UNIDECA) the LH/hCG receptor content remained after d 10 on very low luteolytic level. In control gilts the receptor content of follicular walls was higher during proestrus and estrus compared to postestrus. Because of the absence of follicular growth no follicular receptors were determined in GnRH-A gilts.

The highest estrogen receptor level was detected in endometrium on day 10 and in myometrium on day 20 of estrous cycle, respectively. Compared to normal cyclic changes in control groups (CONT, UNICONT) the estrogen receptor level in GnRH-A treated gilts remained on high values starting at d 15 after GnRH-A.

Maximum endometrial progesterone receptor content was observed between proestrus and early postestrus (day 20 and day 5). No differences were found between gilts of GnRH-A treated and control gilts (UNIDECA, UNICONT).

#### Discussion

Gonadotropin deprivation results in an impairment of reproduction. Hypophysectomy, hypophysial-stalk transection and active immunization of gilts against GnRH induce acyclicity, suppress gonadotropin and estradiol concentrations and cause atrophy of the ovaries (Anderson et al., 1967; Kraeling et al., 1974, 1986; Esbenshade & Britt, 1985). The application of longtime active GnRH agonists and antagonists causes similar but reversible effects (Brebion & Cagnie, 1989; Crowley, 1992; Schneider et al., 1992b; Trimino et al., 1993).

Irrespective of the day of estrous cycle of gilts GnRH-A results in a desensitization of the pituitary and in a decline in circulating LH and steroids. GnRH-A abolished the pulsatile secretion of LH. The basal LH level was not influenced by GnRH-A similarly to the results in prepubertal bulls, ewes and sheep fetuses (Ronayne et al., 1993; Picton & McNeilly, 1991; Brooks & McNeilly, 1992) and in contrast to the results of Roberts et al. (1989) and McNeilly et al. (1992) in postpartum suckled beef cows and in ewes. In cyclic gilts the basal LH concentration was influenced by the day of oestrous. Corresponding to the results of Flowers et al. (1991) there was an increase of LH pulse frequency and a decrease of pulse amplitude during the follicular phase in cyclic gilts.

LHRH bolus injections stimulated a slight increase of LH level between 0.62-1.62 ng/ml only on d 28 after GnRH-A in 4 of 15 gilts. This demonstrated the reversible secretion and/or synthesis of LH at the end of the 28 day down regulating effect of the GnRH-A formulation. Similar to the results in ewes (McNeilly et al., 1992) the development and maintenance of corpus luteum related to progesterone levels and endoscopical observations was not influenced by administration of GnRH-A both in preovulatory and midcyclic gilts.

According to the level of estradiol, remaining during the whole experiment on basal level there were no cyclic changes or elevation indicating active follicular development. Results of repeated laparoscopy confirm the ascertainment of suppressed follicular development. No ovulation and only a small number of follicles 3-5 mm between d 12 and d 32 were observed. Porcine luteal LH/hCG receptors doubled between day 6 and day 10 of the estrous cycle and decreased between day 12 and 14 (Ziecik et al., 1980). The highest LH/hCG receptor content was detected in our experiment on days 5 and 10 of oestrous cycle. The elevated receptor levels in gilts 5 days after GnRH-A administration may be a short-time answer of corpora lutea to the flare-up gonadotropin release stimulus.

Deaver & Guthrie (1980) demonstrated increased endometrial cytoplasmatic estrogen receptors after the onset of oestrous to a maximum on day 10. Kozirowski et al. (1984) found an increase at the follicular phase when plasma concentration of estrogen was high. According to the results of Rexroad & Guthrie (1985) we detected the lowest level after luteolysis and the highest during the luteal phase. GnRH-A treatment increased the amount of unoccupied estrogen receptors.

The progesterone receptor content in uterus showed the same pattern as in oviduct (Stanchev et al., 1985). The lowest amount was estimated during luteolysis.

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**Control of Reproduction with a Progestagen - Altrenogest (Regumate) in Gilts and at Weaning in Primiparous Sows : Effect on Fertility and Litter Size**

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During the two past decade, the industrialization of pig production and the changes in management of sows in France have increased by 7 piglets the annual numerical productivity of sows and reached a mean rate of 22.5 piglets weaned per sow in 1992 (Dagorn, Vaudelet, 1993). France, United Kingdom, Denmark and Netherlands have the best technical results of reproduction of the countries of European Communities (Teffene, 1993).

The French pig breeders are managed in a "all in - all out" system to control the introduction of animals and maintain homogeneous groups of sows. But, in such a system, a strict control of reproduction is necessary.

Sows in commercial herds last, on average for 4 litters before they are culled. For every sow that is culled, a gilt must enter in the farrowing groups. The annual culling rate is about 50% in the French Recording Programme (Badouard et al, 1990). It means that for a unit of 100 sows, about 120 gilts must be purchased or home bred each year (2.3 farrowings per year). For every batch of matings (every 3 weeks) about 10 gilts must be ready to mate and this means that each batch is composed of about 25 % primiparous sows.

*The main reproductive problems and the different solutions to control oestrus in the sow*

The appropriate age to mate a gilt depends mainly on age at puberty. Environmental and management conditions influence the timing of puberty. At around 5 months of age, the presence of the male associated with " stress " factors (transportation or transfer or regrouping gilts) allows synchronization of oestrus within a month for 85% of the crossbred gilts (Signoret et al., 1990). The variability of return to oestrus raises a problem in herds organized into groups of farrowings every 3 weeks.

The oestrous cycle may be altered by inducing regression of corpora lutea or by suppressing ovarian follicular activity to delay oestrus. In the cyclic gilt, prostaglandin  $F_{2\alpha}$  or its analogues did not induce luteolysis when given earlier than Day 12 of oestrus cycle (Guthrie & Polge, 1976) and cannot therefore be used to synchronize oestrus in cyclic gilts. Several orally active progestational agents have been examined in pigs; (reviewed by Webel, 1978). One of the most promising compounds - Methallibure - (Polge et al., 1968) is withdrawn from market in Europe in 1973 because of its teratogenic effect and this has led to a resurgence of interest in progestagens. The general schedule for the use of progestagens has been described (Martinat - Botté et al., 1977). The orally active progestagen Altrenogest or Regumate resulted in satisfactory control of reproduction (Webel, Day, 1982; Martinat - Botté et al., 1985).

After a lactation period of 4 weeks, oestrus usually occurred within one week. The weaning to oestrus interval is longer in primiparous sows than in multiparous sows. Fertility of such females with a delayed oestrus is 10% lower than in sows having a normal oestrus after weaning (Martinat - Botte et al., 1985). No satisfactory results have been obtained in France with an hormonal treatment (PMSG, hCG) due to variability between herds (Martinat - Botté et al., 1975). The effects of a progestagen treatment, in particular Regumate at weaning, have been studied to control the oestrus in primiparous and multiparous sows (Boland, 1983; Kirkwood et al., 1986; Peters, Baker, 1992; Stevenson et al., 1985; Martinat - Botté et al., 1985; Kirkwood, Aherne, 1988).

#### *Use of Regumate for reproductive management of gilts*

Regumate suppresses follicular maturation with no detectable effect on lifespan of corpora lutea when fed at the recommended level: 20 mg daily per gilt (Webel, 1978). Each gilt received a daily dose of Regumate in oil for 18 days in the food (European approved treatment; compound packed in pressurized container). The treatment was started irrespective of the gilt's oestrous cycle. By days 4 to 9 after treatment, 95% of the gilts exhibited oestrus. Synchronization of oestrus is more affected by genotype than by management. A close synchronization is observed in crossbred gilts compared to Large White gilts (Martinat - Botté et al., 1985). The differences observed between herds are explained partly by the fact that some gilts are not pubertal at the beginning of the treatment. Regumate is ineffective in non-pubertal gilts (Martinat - Botté et al., 1982).

A preliminary survey of field use suggested that the treatment resulted in good fertility and also increased litter size (Martinat - Botté et al., 1989). A carefully controlled experiment has been undertaken with crossbred gilts to test this hypothesis. After control of puberty, crossbred gilts were randomly distributed into two groups: a control group and a treated group receiving 20 mg of Regumate daily for an 18 day period. Gilts of both groups were inseminated at the 2nd oestrus (double A.I. at 24h intervals, 6 billion spermatozoa / A.I.).

Regumate treatment gives a high farrowing rate (control: 80.8%, n=281; treated group: 88.4%, n=244,  $p < 0.05$ ) and also increased litter size by 0.5 piglet (control: 9.1; treated group: 9.6,  $p < 0.05$ ), (Martinat - Botté et al., 1990).

We have demonstrated that the ovulation rates increased in the 2nd oestrus after Regumate treatment: 15 (median) vs 14 for the controls (2nd oestrus,  $p < 0.02$ ). One more corpus luteum on average was primarily responsible for the larger litter size observed after Regumate.

#### *Use of Regumate at weaning in primiparous sows*

Recently, we have tested, at the time of the weaning, the efficacy of two Regumate treatments in primiparous sows compared to a control group on the synchronization of oestrus and the reproductive performance.

A total of 654 females belonging to the same farm were dispatched into 3 groups: control sows without any treatment (control), sows treated with Regumate during 3 days - treatment starting at the day of weaning (RU 3d) and sows treated with Regumate during 5 days - treatment starting at 2 days after weaning (RU 5d). The females were inseminated at the 2nd oestrus: double A.I. at 24 h intervals, 6 billion spermatozoa / A.I.. The mean duration of lactation was 27 days. The experiment lasted during three years.

The percentage of females being in oestrus between D4 - D7 (D0 : end of treatment or weaning) was higher for the treated groups : RU3d - 83.4%, RU5d- 82.4% compared to controls - 79.6%. The distribution of the onset of oestrus differed significantly between the treated groups and the control group ( $p < 0.04$ ). The percentage of primiparous sows not coming in oestrus varied from 2.8% to 6.8%. Ovulation rate and development of embryos have been observed for pregnant primiparous sows culled precociously 3rd, 6th and 10th of gestation ( $n=41$ ). The ovulation rate did not differ between groups: 17.2, 17.9, 16.6 corpora lutea in mean respectively for control, RU3d , RU5d groups. The quality of ovulation has been characterized by the absence or presence of cystic follicles or hemorrhagic corpora lutea. This parameter - quality of ovulation - was expressed in percentage of the females with only corpora lutea on the two ovaries. A better quality of ovulation was noted for RU3d (78%) compared to the two other groups (46% - RU5d, 60% - control, not significant).

At the third day of gestation, a significant difference for the embryo development between treatments was noted: 91.6% of the embryos collected from sows treated during 3 days were at the 4 cells stage while only 30 and 43% of embryos collected from sows treated 5 days or control were at the same 4-cell stage. At day 10 of gestation, due to a large variation within each sow, the differences noted for the mean diameter of embryos were not statistically different between groups.

The farrowing rate was significantly ( $P < 0.05$ ) higher for the RU3d group. Litter size increased for the treated groups as compared to the controls (Table 1). However, the differences were not significant. The same trend was noted for the criteria : live born piglets and weaned piglets. Primiparous sows from RU3d group has a better productivity ( $p < 0.04$ ) than the other groups, nearly one piglet more (Table 1).

**Table 1:** Effect of Regumate on farrowing rate, litter size and productivity at weaning in primiparous sows

Group	N	Farrowing rate (%)	Litter size *	Productivity**
Control	201	76.1	9.8	7.2
RU 3d	202	82.2	10.1	8.0
RU 5d	207	71.5	10.4	7.0

\* total born piglets \*\* number of live born piglets per treated sow.

### Conclusion

Under conditions of practical pig production, an efficient control of oestrus is obtained with Regumate in puberal gilts. An improved farrowing rate together with an increase in litter size is expected. These combined effects represent an improvement of around one piglet for the first litter. The Regumate is used in France and has considerably facilitated the introduction of the gilts into the breeding herd. Actually, half of the gilts are joining the groups of farrowings through a Regumate treatment.

In primiparous sows, Regumate administered during 3 days after weaning has permitted to obtain the best results in terms of synchronisation of oestrus, quality of ovulation, farrowing rate, litter size and productivity (increase of around one piglet). Actually, it is not allowed in France, for sows at weaning.

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### **Ultrasonography of Pig Ovaries: Benefits in Research and on Farms**

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#### **Introduction**

Two-dimensional ultrasonography to diagnose reproductive (dys)functioning in farm animals has first been used for pregnancy diagnosis in horses (Palmer and Driancourt, 1980) and later in the other farm animals, including pigs (see Kähn, 1992). Since 1989, ultrasound is used to diagnose (dys)functioning of pig ovaries. Weitze et al. (1989, 1990a,b) used transcutaneous ultrasonography to assess the time of ovulation and Soede et al. (1991, 1992, 1993) used transrectal ultrasonography to assess the time and duration of ovulation. The use of ultrasonography of pig ovaries may be benefited from in research and on farms, as will be discussed below.

#### **Material and Methods**

The ovaries can be visualized using either transcutaneous or transrectal ultrasonography. Transvaginal ultrasonography, which is used in human, is not an option in pigs due to the anatomical position of the ovaries.

- Transcutaneous ultrasonography. The ovaries are found dorso-cranial of the hind knee, a few centimeters ventro-cranial of the hipbone. To visualize them with ultrasound, the transducer has to be placed just above the udder, scanning upwards. The ovaries can be found a few centimeters cranial of the bladder which serves as a recognition point.
- Transrectal ultrasonography under manual guidance. The ovaries are found at 30-40 cm cranial of the anus, ventro-cranial of the rectum. A lubricated glove removes the faeces and brings in the transducer. Again, the bladder may serve as a point of recognition.

The major disadvantage of transcutaneous ultrasonography (see table 1) is that it requires a lot of experience and, furthermore, that the image is less clear (skin) and less stable (sow movements) which makes it more difficult to interpret the images. The major disadvantage of transrectal ultrasonography is the fact that it can not be applied in gilts (pelvic bones too narrow). The choice for either of the two methods should therefore depend on the goal of the experimenter/practitioner.

Table 1. Positive (++) and negative (--) aspects of transcutaneous and transrectal ultrasonography in visualizing pig ovaries.

Transducer		Transcutaneous	Transrectal (manually)
frequency		5 MHz	5-7.5 MHz
type		sector/linear	sector/linear
applicable in	gilts	++	--
	sows	±	++
intestines block	sight	--	+
image quality		±	++
experience needed		--	+
follicles:	recognition	+	++
	count	--	++
corpora lutea:	recognition	±	++
	count	--	--

#### *Applications in research*

In research, Weitze et al. (1989, 1990a,b) used transcutaneous ultrasonography to assess the time of ovulation and Soede et al. (1991, 1992, 1993) used transrectal ultrasonography to assess the time and duration of ovulation. In the future, it will undoubtedly also be used to assess follicular development, as it is done in cattle and horses (see Kähn, 1992), including research concerning possible influences on follicular development.

#### *Time of ovulation*

In our research group, the time of ovulation was assessed in 151 multiparous sows during the first oestrus after weaning. In these sows, onset of oestrus occurred between 4 and 7 days after weaning. Oestrus was checked every 8 hours with a vasectomized boar and lasted between 24 and 88 h (see Figure 1). Ovulation was checked every 4 hours using transrectal ultrasonography and occurred between 16 h and 58 h after onset of oestrus (at on average  $35 \pm 8$  h, ranging from 10 to 58 h). From the figure, it is clear that ovulation occurs at a later time after onset of oestrus in sows with a longer duration of oestrus. More specifically, ovulation takes place at  $72 \pm 15$  % of the oestrus (range 39 to 133%) (C.C.H. Wetzels et al., unpublished results). Possible causes for the variation in the duration of oestrus and the timing of ovulation are investigated (Wagner-Rietschel, 1991; Soede et al., 1994). Assessment of the time of ovulation is furthermore of importance in research concerning e.g. relationships between hormone profiles and ovulation (Soede et al., 1994) and insemination strategies (Weitze et al., 1990a,b; Wetzels, 1994).

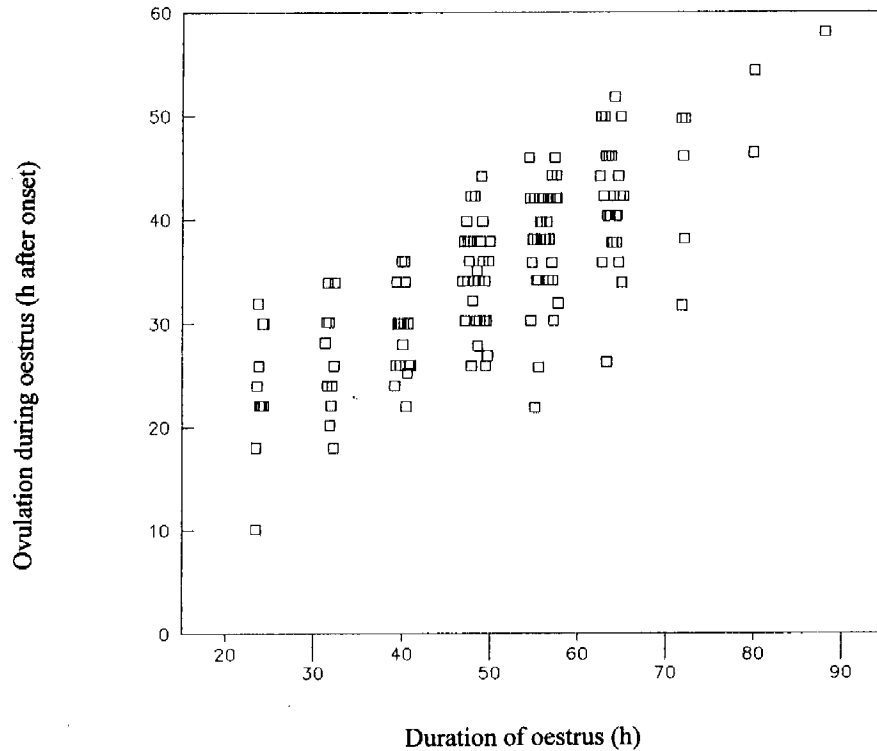


Fig. 1: Relation between the duration of oestrus and the time of ovulation in 151 multiparous sows. (C.C.H. Wetzels, unpublished results).

#### *Duration of ovulation*

Variation in the rate of embryo mortality is a major cause of differences in litter size. From 1980 onwards, evidence accumulated that embryo diversity around day 11 of pregnancy causes the death of the lesser developed embryos in the litter (e.g. Geisert, Pope, 1990). Pope et al. (1990) suggested that the duration of ovulation may be one of the important causes of embryo diversity. In our research group, the number of follicles was counted on both ovaries at 30 min intervals with ultrasound. In sows in which the duration of ovulation varied between 1 and 4 h, embryo diversity at Day 5 of pregnancy was not related to this duration, neither in spontaneously ovulating sows (Soede et al., 1992) nor in sows with induced ovulation (Soede and Kemp, 1993). Besides consequences of variation in the duration of ovulation, assessment of the duration of ovulation naturally allows for research concerning causes of this variation. For example, indications exist that under stressful conditions, sows may have a longer duration of ovulation (Soede et al., 1992); subsequent consequences for embryo diversity are studied.

*Applications on farms with reproductive problems*

Ultrasonography provides the opportunity to visualize the ovaries of sows very clearly and does not appear to have any adverse effects on fertility or other processes (Soede and Kemp, 1993). This offers a number of opportunities for the use of transrectal ultrasonography as a diagnostic tool at farms with fertility problems, for example with respect to oestrus detection, farrowing rate and litter size. As stated above, transrectal and transcutaneous ultrasonography both have their disadvantages which limits their use.

*Oestrus detection*

When a large number of sows do not appear to be cyclic, an examination can be carried out using transrectal ultrasonography to ascertain whether the animals are cyclic or not. If they appear cyclic, they may have only a very short oestrus, a silent oestrus or a lactation oestrus or oestrus detection may not be performed properly. Sows that are not cyclic may have either inactive ovaries or cystic ovaries. All these pathological stages of the ovary can be traced with ultrasound. In some animals it may be necessary to scan more than once to make the right diagnosis; e.g. an inactive ovary resembles an ovary at day 17 of the oestrus cycle. Using transcutaneous ultrasonography, animals will probably have to be scanned more than once to make the right diagnosis because corpora lutea are difficult to identify using this method.

*Farrowing rate*

Low farrowing rates are often the result of an insemination strategy in which unfavorable intervals have arisen between insemination and ovulation. Just recently, using transrectal ultrasonography to detect ovulation, we have found that insemination must take place between 0 and 24 hours before ovulation in order to obtain a good fertilization result. (C.C.H. Wetzels, unpublished results). In order to be able to check this interval on farms, it is necessary to scan sows both at insemination and 24 hours later. Sows which have already ovulated at insemination were inseminated too late and sows that have not ovulated 24 hours after insemination were inseminated too early. Due to the enormous variation in the duration of oestrus on one farm (see figure 1 in which gilts are not even included), it is impossible to inseminate all animals at the right time. However, if either too early or too late insemination appears frequently, an attempt can be made to improve the insemination strategy, the oestrus detection or both. It is important to note, that the average duration of oestrus may vary extremely between farms, from 38 h to 69 h (25 farms; Groenland, 1991).

*Litter size*

As already stated above, litter size is predominantly determined by the level of embryonic mortality and the number of ovulations. Besides these, litter size may also be disappointing because of low fertilization rates. This is often caused by an incorrect moment of insemination (see 'farrowing rate'). In case of disappointing litter sizes, it may be worthwhile to count the number of pre-ovulatory follicles by transrectal (and not transcutaneous) ultrasonography during oestrus, as a measure for the number of ovulations. Repeated counting of the follicles during oestrus revealed in 56 ovaries that the follicle count was exactly the same as the number of ovulations (corpora lutea at slaughter, 5 days after ovulation) in 50% of the ovaries, differed one in 27% of the ovaries and differed two in 18% of the ovaries and more than two in 5% of the ovaries (Soede, 1992). Depending on the outcome, management may be changed either to increase the number of ovulations or to reduce embryo mortality.

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### **Reproductive Management of Middle Sized Pig Farms**

Holtz, W.

The average herd size of a West-German pig breeding farm is less than 60 sows. The ex-socialist part of Germany is left out of consideration because agricultural restructuring has not yet taken final shape. Disregarding the small number of herds with more than 100 and the considerably larger number with less than 10 sows, the average middle sized pig farmer owns between 40 - 80 sows. Typically, these piggeries are part of a mixed-farming or part-time-farming enterprise and they are often the domain of the farmer's wife.

Usually, groups of sows rotate between three separate housing units: Weaned sows and gilts that are to join the breeding herd are kept in the mating quarters ("eros center"). After pregnancy is established they are moved to the waiting quarters and, eventually, to the farrowing quarters ("maternity ward"). In addition, there is usually a climatized room for the piglets from weaning to 25 - 30 kg, and accommodation for the prepubertal replacements.

Maximum attention is required while the females are in the mating quarters. This institution was missing in the large pig herds of what used to be the socialist countries of Eastern Europe. In these countries hormones were used to control the cycle as a standard procedure. Under present-day conditions, economic and ethical considerations speak against large scale routine use of exogenous hormones. In the "eros center" the females are kept in close proximity to a sexually mature male. The vicinity of the boar enhances the onset of estrus and facilitates estrus detection. It is more efficient to have the females in the position to approach the boar's pen rather than keeping them in crates with the boar having to search for estrous animals. Recently electronic aids have been designed, by which frequency and duration of a sow's visits to the pen of the boar may be recorded. With group-housed females equipped with electronic identification (chips), this concept will, in all probability, gain in popularity. If no boar is available, the stockman has to resort to visual observation of estrus symptoms, supported by manual and olfactory (synthetic boar taint) stimulation. A minimum of 5 - 10 min has to be invested per sow. Best results are accomplished if heat-testing is conducted twice daily. Careful record keeping and the use of an appropriate computer program help save time and increase efficiency. None of the available devices for recording pH or electric conductivity of vaginal mucus have proven to be effective in predicting the optimal time for insemination.

If there is a need for estrus induction, PMSG or PMSG combined with hCG ("PG 600") may be injected. Usually the treatment is limited to prepuberal gilts or weaned first litter sows that are to be synchronized with groups of weaned sows in all-in-all-out management systems. The progesterone Altrenogest may be administered orally to cycling sows in order to temporarily suppress ovarian activity. This is, however, labor intensive and expensive and, therefore, rarely applied.

Artificial insemination (AI), performed by the farmer, is increasingly used as the major means of impregnating sows. AI was initially used in the very small breeding herds of Southern Germany. With the tendency to have groups of sows in heat at the same time and gaining

importance of cross-breeding programs, AI becomes indispensable. Semen is mailed by AI stations upon telephonic request. When stored at 15 - 18°C it retains its fertilizing capacity for at least 3 days. Therefore, most farmers apply a double insemination at a 12 - 24 h interval. Even farmers who choose to have their sows mated make use of AI to back up the breeding male in situations where too many females come into heat or the boar fails to serve. For natural mating, one boar is needed for 25 females.

Females remain in the mating quarters until pregnancy is established. This is accomplished by closely monitoring for signs of estrus 3 weeks after insemination. Most farmers also pregnancy-test their animals with pulse mode ultrasound equipment. This technique is easy to conduct and provides reliable results between 4 - 12 weeks of pregnancy. Alternatively, Doppler ultrasound equipment may be employed. The Doppler system permits reliable identification of non-pregnant animals in time for reinsemination at the first return-estrus. It does, however, require considerably more time and experience and the equipment is more costly.

Once pregnancy has been established, animals are moved to the waiting quarters, where they are usually kept in groups of 5 - 15 under fairly extensive feeding and management conditions. Beginning 3 - 4 weeks before they are due to farrow, the level of nutrition is gradually increased. One or two weeks before the expected time of farrowing, sows are wormed, scrubbed down with soap and water containing a parasiticide and moved to the farrowing quarters where they are kept in farrowing crates. About 3 days before expected date of farrowing, the feed supply is drastically restricted. Compounds rich in crude fibre components (e.g. bran) are increased and Epsom or Glauber salt may be administered. This serves the purpose to prevent constipation and, as a frequent sequel, the post-partum complication of metritis, mastitis and agalactia, the "MMA-Syndrome". In many herds the stockman attempts to supervise parturition. In doing so, periparturient losses may be cut by 0.5 - 1 piglet per litter. Parturition induction as a means to improve efficiency of parturition supervision and equalize litter size by cross-fostering is applied by a number of farmers. This is accomplished by injecting a PGF<sub>2α</sub>-analog at any time after day 111 of pregnancy. Parturition will commence about 24 h after injection. The effectiveness may be improved by injecting a beta-receptor-blocker about 20 h after the prostaglandin treatment.

On each of the first three days following parturition, conscientious farmers record the rectal temperature of their sows. The incidence of MMA is highly variable from herd to herd. It may cause substantial economic loss due to starved or undernourished piglets. If the rectal temperature increases beyond 39.3°C, antibiotic treatment is indicated even if there are no clinical signs of mammary inflammation or lack of appetite. If clinical signs are evident, antibiotic treatment may be supplemented by corticosteroids and oxytocin.

On most middle-sized pig farms the weaning age averages 4 weeks. Weaning is delayed if the piglets have not reached 6 kg. About 3 weeks postpartum uterine regression has been completed and, under optimum management conditions, sows will return to estrus within 4 - 8 days. On some farms first-litter sows receive a gonadotropin injection at the time of weaning. This is not necessary if gilts are first mated at a body weight beyond 120 kg, and if the feeding level during the lactation period is such that the sows are weaned at a reasonable body conformation. Post-weaning flushing may be effective with first litter sows or overly lean animals but must be discontinued upon insemination to avoid enhancement of embryonic mortality.

Health precautions such as rigorous disinfection programs for buildings and equipment, advance confrontation of replacement gilts with the germ environment prevailing in the herd, observation of a quarantine period prior to introducing breeding stock from other herds,

routine vaccinations against parvovirus and a number of other diseases are of utmost importance but will not be covered in this paper.

The trend in piglet production is towards larger units. These have obvious advantages, but also disadvantages such as large capital investment, the need for hired labor, a high risk element (epidemics, market fluctuations) and environmental problems with the disposal of animal wastes. These problems are less pertinent in small and medium size herds. There is also the advantage of the individual attention and care sow and piglet are getting. As a result health and reproductive performance are superior and, in particular, piglet losses are reduced.

The middle sized pig farm has its place as part of a family farming enterprise. It has a chance to survive as long as it is managed according to up-to-date technology and integrated into a cooperative or commercial organization that takes care of genetic, organizational and marketing concerns.

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### Artificial Insemination in Pig Farms

Weitze, K.F. and Waberski, D.

#### *1. Storage time of liquid boar semen and time interval between AI and ovulation as influence factors upon fertilization in gilts*

In pigs, high variation is seen in the duration of estrus and the time of ovulation. This is one of the factors not related to semen quality, which influences the results of field insemination trials. 81 gilts were experimentally inseminated after detection of standing heat by a teaser boar to determine the influence of the ovulation time on the fertilization capacity of liquid boar semen stored up to 5 days. Fertilization rates based on Day-2 and Day-4 embryos, and the number of accessory spermatozoa per zona pellucida did not differ significantly between semen stored for 0 to 48 h and 48 h to 87 h in gilts ovulating within 12 hours after insemination. Gilts with an interval of 12 to 24 hours between AI and ovulation had lower fertility results using semen stored for more than 48 h. A further decrease was observed when semen storage exceeded 87 h in those gilts ovulating later than 24 h after insemination. Therefore, a potentiating effect of *in vitro* aging during semen storage and subsequent *in vivo* aging within the sows genital tract can be suggested. The results are of practical importance for the AI management in breeding sows (Waberski et al., 1994).

#### *2. The effect of insemination time relative to ovulation on fertility after use of liquid and frozen boar semen*

76 and 80 gilts were inseminated before, during and after ovulation, using fresh and frozen semen (experiments 1 and 2). Additionally, the extent of embryonic loss in the first 28 d of pregnancy following pre- and postovulatory inseminations with fresh semen was studied in 24 gilts (experiment 3). In gilts inseminated with fresh semen more than 12 h before ovulation significant lower fertility results were achieved compared to inseminations realized 12 - 8, 8 - 4 and 4 - 0 h before ovulation (55.8% versus 96, 85.5 and 93.4% respectively). In early postovulatory inseminations (0 - 4 h), 92.5% of the oocytes were initially fertilized, whereas later inseminations (4 - 8 and 8 - 12 h after ovulation) revealed a significant decrease. Using frozen - thawed semen. AI performed 4 - 0 h before ovulation showed comparable results (88.1%) whereas earlier AI (8 - 4 h before) or AI after ovulation (0 - 4 h) was characterized by a significant decrease of fertility to 54.9 and 50.0% respectively. Thus, the "window" for optimal fertility results using frozen semen seems to be much shorter than after use of fresh semen. In the gilts slaughtered 28 d after postovulatory inseminations with fresh semen a significant decrease of the number of fetuses was found compared to inseminations performed before ovulation (7.7 versus 10.1). It can be assumed that increased embryonic mortality is responsible for reduced litter size after postovulatory fertilization of (aged) oocytes (Waberski et al., 1994).

*3. The onset of heat after weaning, heat duration and time of ovulation, three important influence factors in AI in breeding sows*

Transcutaneous sonography of the ovaries to detect ovulation was used in 483 sows of a breeding herd. Estrus was checked three times daily using a teaser boar to determine begin and end of acceptance. Successful ovulation detection was possible in 427 sows. Over 70% of the sows showed a standing heat period between 2 and 3 days, whereas two subpopulations of sows had shorter (less than 2 days; 14.3%) or longer heat periods (4 days and longer, 12.7%). The distribution of the time of ovulation (interval between heat onset of detection of ovulation) shows a similar distribution. More than 80% of the sows had ovulated between 32 and 56 h after heat detection. Interesting is a subpopulation of 7.5% of sows, which already had ovulated within 24 h after heat detection. Another population of approximately 9% of animals ovulate very late between 64 and 80 h after heat onset. To make these informations useful for practical work in a herd, the time relationship to weaning must be considered. After weaning Thursday morning, already more than 80% of the sows show standing heat symptoms at Tuesday evening (day 5 after weaning), but scarcely 3.6% of the animals had ovulated at this moment. Ovulations are concentrated on Wednesday and Thursday. At Wednesday evening nearly 50% of the sows had ovulated, whereas further 28.8% ovulated during Thursday. The data show clearly the time shift between onset of heat and ovulation which seems to be one important reason for low fertility rates in consequence of too early inseminations more than 24 h before ovulation. Statistical calculations of the results of this study show a significant interdependence of the interval between weaning and heat onset, heat duration and interval between heat onset and detection of ovulation. For practical use the following recommendation can be established. Sows coming in heat early after weaning (within four days) have longer heat durations than sows coming later in heat and show longer intervals between heat onset and ovulation than sows with short estrus periods. Sows coming late in heat (six days and more after weaning) had predominantly short heats. This group includes 7.5% of sows, in which ovulation already occurred within the first 24 h of heat. Therefore, in sows entering heat early after weaning the first insemination should be done at the earliest after 24 h. Special attention has to be paid to the second and third day of heat, in which ovulation is to be expected and inseminations therefore should be repeated. In sows with an average weaning - heat onset interval the first insemination should be made 24 h after heat detection and repeated 12 h later. Sows with late heat onset generally have short heat periods and ovulate early after heat onset, an insemination as early as possible is necessary. A second insemination is usually not needed (Weitze et al., 1994).

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### Physiology and Preservation of Boar Semen

Rodriguez-Martinez, H. and Eriksson, B.

#### Introduction

The use of frozen boar semen for AI is still very limited worldwide, mainly due to the lower fertility of the thawed semen. So far, no reliable cryopreservation procedure has been designed and most attempts had been empirical. Among the missing points in the design of protocols for semen handling are the reasons behind the structural and functional alterations of the spermatozoa which convey a reduction of their fertilizing capacity. Further, our ability to use proper *in vitro* systems to assess the functional status of spermatozoa before and after cryopreservation is yet limited. The present mini-review describes some physiological aspects of boar spermatozoa of major importance for fertility in relation to semen cryopreservation.

#### *The mature spermatozoon*

Spermatozoa develop during a 2 months-long process in the testis (spermatogenesis) from where they are delivered to the epididymis. During their 2 week-long journey through the epididymal duct, the spermatozoa undergo morphological, biochemical and physiological modifications to finally acquire progressive motility and the ability to fertilize ova, as well as they are prevented to undergo acrosomal reaction within the duct (rev. by Rodriguez-Martinez 1991). The spermatozoa are further modified through their contact with seminal plasma and the uterine-oviductal environment, events often grouped under the name "post-testicular sperm maturation" (Crabo 1985) and considered essential for fertilization through the induction of the capacitation process (Gadella 1994). The mature boar spermatozoon is a highly specialized and polarized cell whose plasma membrane can be divided into several functional, structural and biochemical distinct domains, relevant for the series of events leading to fertilization (Aloia et al. 1988). The sperm membranes (plasma, mitochondrial, acrosomal) are unique aggregates of lipids and proteins, assembled during spermatogenesis and modified during epididymal transit, storage and ejaculation. The two main functions of the acrosomal membrane segment (anterior sperm head) are to bind to the zona pellucida and to fuse with the underlying acrosome, thus allowing the release of the acrosome contents and the presentation of the inner portion of the acrosome membrane as a new surface. Any modification of this membrane domain will block the activities of the other domains and ultimately convey to the infertility of the spermatozoon (Cardullo & Wolf 1990).

#### *Preservation of boar semen*

Boar semen can be preserved *in vitro* in liquid or deep-frozen forms. For liquid preservation, boar semen is isothermally diluted with special dilution buffers (ex. Beltsville Thawing Solution, BTS, among many others) and cooled to 16-20° C. This liquid-preserved semen is usually employed within 48 h in 80 ml-doses each containing 3 billion sperm, yielding not only farrowing rates and litter sizes comparable to natural mating, but also increasing the effectivity of the collected ejaculate by 10-fold or more. For cryopreservation, boar semen is first diluted to wash out most seminal plasma, cooled, mixed with cryoprotective diluents

(holding low concentrations of glycerol) in one or several steps, further chilled, packaged in plastic containers, and frozen with liquid nitrogen in programmable freezers following defined cooling rates (Bwanga 1991).

Several research groups have developed both their own diluents and procedures for freezing of boar semen (Polge et al. 1970, Graham et al. 1972, Crabo & Einarsson 1971, Visser & Salamon 1974, Pursel & Johnson 1971, Westendorf et al. 1975; Larsson & Einarsson 1976, Paquignon & Courot 1976). Freezing semen in pellet form had been very successful, but pellets have however, disadvantages in identification, storage, thawing, and in the need of large numbers of pellets per insemination dose. Maxi-straws (5-6 ml), although conveying most of these problems, have the drawback of being cryobiological unsuitable, due to their large section and volume, that impairs both proper freezing and thawing. Flattened straws (Weitze et al. 1988) and plastic bags (Bwanga et al. 1991) appear to be more convenient since they can include both nearly the same surface:volume ratio as mini-straws. As it remains a major constrain for AI-practice, attempts have been made at our department to develop alternative packaging methods for large concentration/volumes of semen (5 ml volume plastic bags), that is to freeze enough numbers of spermatozoa to fill a single AI-dose with acceptable post-thaw fertility (Larsson et al. 1976, Bwanga et al. 1991, Mwanza & Rodriguez-Martinez 1993, Karosas & Rodriguez-Martinez 1993). Nevertheless, the physical stress associated with cryopreservation and thawing substantially diminished the survival and fertilizing ability of the thawed spermatozoa (Mwanza & Rodriguez-Martinez 1993, Ortman & Rodriguez-Martinez 1993). Studies are being conducted that aim to improve the quality of frozen/thawed boar semen in single AI-dose commercial containers (Eriksson & Rodriguez-Martinez unpublished).

#### *Effects of cryopreservation onto boar spermatozoa*

The low viability of frozen-thawed boar spermatozoa seems to be due to their considerable sensitivity to cold shock, with plasma membrane damage, modifications in the ion balance and even DNA alterations, that could explain why fertilization and early embryo development are impaired after insemination (Watson & Plummer 1985). When a biological membrane is cooled, its components can become reorganized through the aggregation of constituent non-bilayer lipids and a protein redistribution (Hammerstedt et al. 1990). The lower the temperature the greater the proportion of lipids that undergo a phase transition (Watson & Plummer 1985) and are released (predominantly phospholipids) from boar sperm membranes (Pickett & Komarek 1967). Cold-induced changes in sperm membrane organization have been demonstrated through scanning electron microscopy of freeze-etched samples (De Leeuw et al. 1990). The microscopy revealed that particles aggregated in the plasmalemma upon cooling to 5-0° C, particularly in the head region. It was also found that the phenomenon could be reversible by rewarming.

#### *Quality sperm parameters of importance for fertility*

Among the *in vitro* methods developed to evaluate semen quality (i.e. sperm integrity and viability) and predict the fertilizing ability of a given sample, post-thaw motility and amount of normal apical ridges (NAR's) are the most widely used (rev. by Larsson 1985). However, these *in vitro* methods do not provide accurate assessments of the fertilizing capacity of frozen-thawed boar semen (rev. by Woelders 1991). NAR's assessment is a rough method which can not detect any subtle changes in the acrosome functionality. Fluorochromes have commonly been used to assess the ability of the sperm membrane to either exclude dyes (ex. eosin) or to show their permeability. Garner et al. (1986) described the use of two fluorescent probes, carboxyfluorescein diacetate (C-FDA) and propidium iodide (PI), in studying the membrane integrity of spermatozoa of domestic species. This procedure can be used with quantitative flow cytometry (Almlid et al. 1989) or with fluorescent microscopy in smears of

living, formalin-immobilized spermatozoa (Harrison & Vickers 1990). Since simultaneous evaluations of acrosomal status and cell membrane integrity are advantageous when developing a successful cryopreservation method, these supravital fluorescent dyes were used to determine the integrity of the sperm plasma membrane during cooling, freezing and thawing of boar semen in 5-ml plastic bags. Cooling to +5° C significantly affected the permeability of the plasma membrane. By contrast, supercooling (-6° C) during the assayed freezing program did not lead to any further deterioration of the membrane integrity of the spermatozoa. On the other hand, the thawing procedure used, dramatically increased the percentage of spermatozoa with damaged plasma membranes, without being detrimental to motility (Ortman & Rodriguez-Martinez 1993). This reinforces the need of carrying in vitro tests where modifications of the sperm membrane can be accurately monitored throughout, in studies attempting to optimize procedures for freezing and thawing of boar semen.

**Acknowledgements**

This work was supported by the Swedish Council for Forestry and Agricultural Research (SJFR).

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### DNA-Test for Detection of Porcine Stress-Susceptibility

Solti, L. and Bosze, Zs.

The porcine stress susceptibility which is accompanied by halothane sensitivity and inferior pork quality is a good example for disorders that have been studied and are known on gene level. The hypersensitivity for inhalational anesthetics (halothane, isofluran) and for some depolymerizing skeletal muscle relaxants is known in several species, e.g. human, porcine, equine, feline and canine, and often referred to as malignant hyperthermia (MH). As the disorder in the swine can be triggered by stress factors (transport, vaccination) as well, it is known also as porcine stress syndrome (PSS). The economical losses of the meat industry is derived from the phenomenon that the pork of stress susceptible animals is of low quality: pale, soft and exsudative (PSE). Therefore, it is imperative that stress susceptible individuals will not be bred in order to prevent the inheritance of the disorder. Screening of the carrier animals can be performed by halothane challenge test generating symptoms that are the followings: tachycardia, elevated respiration, muscle contractions, circulation insufficiency and cardiogen shock. Typical hyperthermia symptoms are shown only by homozygous (nn) but neither by heterozygous carriers (Nn) nor homozygous resistant (NN) individuals. In contrast to the autosomal dominant human inheritance the defect allows autosomal recessive or co-dominant inheritance in swine. The conventionally used halothane challenge test is time consuming, inaccurate and unable to differentiate the homozygous (NN) and heterozygous (Nn) resistant animals. This means that about 5% of the individuals diagnosed as stress-resistant are heterozygous carriers of the defect and can be verified only by crossing experiments.

Extensive classical genetic studies on stress susceptibility demonstrated that there is a linkage between the halothane and the glucose phosphate isomerase (GPI) loci on chromosome 6p12-q22. This means that GPI locus locating about 0.6 cM from Hal locus is an informative marker that can be diagnosed by DNA probes in good accordance with halothane challenge tests. Due to a slight polymorphism the detection of this marker alone is not always sufficient, therefore, other linkage genes had to be found to diagnose the defect. Some muscle-specific receptors playing a role in initiating the muscle contractions or nerve conduction, e.g. dihydropyridine receptor (DHPR) and ryanodine receptor (RYR) are further candidates for characterizing the halothane gene. During electrophysiological experiments on the sarcoplasmic reticulum, the MH-susceptible animals reacted differently to the same stimulus as did their resistant counterparts which is reflected by a facilitated opening and inhibited closing of the  $Ca^{2+}$  channels.

Genetic and molecular biological trials were conducted and verified that the primary defect of this trait lies in a point mutation on the ryanodine gene coding the  $Ca^{2+}$  release channels in the terminal cisterna of the sarcoplasmic reticulum in the skeletal muscles.

In MH-susceptible pigs the  $Ca^{2+}$  release channel gate opening is facilitated while its closing is inhibited resulting in an elevated  $Ca^{2+}$  release from the terminal cisterna. Both the RYR gene of skeletal muscles and the porcine Hal locus have been localized to the 6p11-q21 region

suggesting that there is a linkage between the malignant hyperthermia and ryanodine receptor gene. Thereafter a point mutation has been identified at the 5'-end of the RYR gene which was always associated with the PSS phenotype. In animals not carrying the defect, the cDNA at the nucleotide 1843 position is C which is replaced with T in malignant hyperthermia founder animals. This substitution is resulting in an exchange of the amino acid sequence of the receptor protein: at position 615 cysteine instead of arginine is found.

It is not yet clear whether the RYR gene acts alone or is interacting with other factors when causing malignant hyperthermia. Supposedly the decreased intraluminar  $\text{Ca}^{2+}$  binding and/or defects of the appropriate  $\text{Ca}^{2+}$  binding receptors play a role in the disorder. The interaction of halothane with cell organelles results in a concentration of narcotics in the muscle cells causing decreased ATP synthesis that can be additional factors of a hyperthermia crisis.

One of the most important breakthroughs in biology was the development of polymerase chain reaction (PCR) which enables to detect minute amounts of DNA. This method amplifies the very low quantity of DNA to a level which can be detected by other, less sensitive assays, such as agarose gel electrophoresis. The basis of PCR is that an enzyme called DNA polymerase is able to extend short DNA sequences (oligonucleotide primers) if they are coupled to a longer DNA strand called template. This enzyme builds always a nucleotide which is complementing to the template to the primer repeatedly as long as the original double stranded DNA is repaired. Thus, the complementing strand of a single-stranded DNA can completely be reconstituted. Recently a heat-stable enzyme from the *Thermus aquaticus* bacteria (Taq polymerase) is used for the PCR assay which is carried out as follows: First the samples are heated to 90°C where the DNA segregates into two single strands (denaturation), then primers are added to the system at 60°C where they are coupling to their complementing counterpart (annealing). The annealing takes place only of the primer that finds the appropriate sequence of the template. In the last phase the Taq polymerase enzyme is building nucleotides to the primer (extension) and reconstitutes the original double-stranded DNA. Repeating this cycle 30 - 50 times, the very small amount of DNA will be amplified to a detectable level.

The availability of PCR enabled to develop and introduce a new test system for the differentiation of PSS carrier and normal animals. During this DNA-based test blood samples of individuals are used for genomic DNA preparation from which the gene sequence carrying the point mutation is amplified by PCR. The end product of PCR is digested by the Hha I restriction enzyme of the recognition site of which it is at the site of point mutation. Digestion of DNA from normal and mutant individuals will result in DNA fragments of different length (restriction fragment length polymorphism, RFLP). As the recognition site of Hha I is the cytosine at nucleotide 1843 position, the enzyme will cut the 134 bp exon into two fragments of 50 and 84 bp. If however, the cytosine at the 1843 position is replaced by thymine in malignant hyperthermia carrier animals, this recognition site is missing and the 134 bp fragment will not be splitted. Accordingly, the gelelectrophoresis will result in two bands in homozygous stress-resistant (NN) animals (50 and 84 bp), three bands in heterozygous stress-resistant (Nn) animals (50, 84 and 134 bp), but one band only in homozygous stress-susceptible (nn) individuals (134 bp). These results are in good accordance with phenotype data gained by the halothane challenge test.

The main advantage of the DNA-based thesis is that they can be carried out from small sample amounts (a few drops of blood or tissue sample) rapidly (PCR) and easily (RFLP). In Southern Germany, Landrace breeds were screened using gene diagnostic methods and grouped as homozygous stress-resistant for breeding, heterozygous stress-resistant for mass production and homozygous stress-susceptible for removal from the livestock. This method

prevented not only from losses due to stress but also reduced significantly the halothane release into the atmosphere and protected the environment.

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## Book Reviews

**G.J. King: Reproduction in Domesticated Animals.** World Animal Science Volume B9. Elsevier Science Publishers B.V. Amsterdam, 1993. 608 S., US \$ 328,25; Dfl. 525,-. ISBN 0-444-89530-2

Analytical assessment of an alternate methodology with potential to enhance fertility or prolificacy requires an extensive knowledge of reproductive biology. Several books on Animal Reproduction are on the market. None of them has ever covered the complete reproductive process in such an excellent way like the new volume of the World Animal Science Series. Experts of different countries like G.J. King, J.C. Meijer, L.-E. Edqvist, G.H. Stabenfeldt, B.P. Setchell, G.R. Foxcroft, C. Fabre-Nys, P. Poindron, J.-P. Signoret, J.J. Parrish, N.L. First, W.W. Thatcher, D.G. Porter, D.A. Croy, R.F. Seamark have contributed in the 19 different chapters. The reader is guided thoroughly through all interesting processes like biology of reproduction, structural development of reproductive organs, hormones of reproduction, endogenous control of reproductive processes, male reproduction, female reproduction, reproductive behaviour, fertilization, pregnancy, parturition, immunoreproduction, biotechnology and reproduction, artificial insemination, embryo transfer, genetics of reproduction, environment and reproduction, nutrition and reproduction, reproduction in poultry and reproductive performance and problems. A great number of very informative figures and tables are shown as explanation to the text. Each chapter is supplemented with the important references. Special information is easily to be found in the subject index. The clear and comprehensible text is easy to read, also for people with less experience in the English language.

The book would be an excellent standard book for all practitioners, scientists as well as students of veterinary medicine or agricultural sciences. Unfortunately, the price of the book is unusually high, a fact which will prevent its world-wide distribution.

D. Rath, Mariensee

**Johannes Richter und Richard Götze: Tiergeburtsilfe.** 4., völlig neubearbeitete Auflage, hrsg. von E. Grunert, Tierärztl. Hochschule Hannover & K. Arbeiter, Veterinärmed. Universität Wien. Verlag Paul Parey, Berlin und Hamburg, 1993. ca. 672 S., 397 Abb. mit 493 Einzeldarstellungen im Text, 2 Farbtafeln und 77 Übersichten. 27 x 18 cm, geb., DM 198,-. ISBN 3-489-53416-6

Vor mehr als 40 Jahren erschien die erste Auflage der „Tiergeburtsilfe“. Die 1978 aufgelegte 3. Auflage war innerhalb weniger Jahre vergriffen, was die Bedeutung des Buches und das Interesse hieran bekundet. Als Gemeinschaftswerk von 23 Autoren unter Leitung von E. Grunert und K. Arbeiter entstand die völlig neu bearbeitete 4. Auflage, die gerade erschienen ist. Der Umfang wurde auf wesentliche Informationen um rund ein Drittel gekürzt, was der Qualität des Buches, das als Nachschlagewerk und Lehrbuch dienen soll, keinen Abbruch getan hat. Im Gegenteil, durch die Einbeziehung neuer Sachgebiete, wie einer intensiven Berücksichtigung der Geburtsilfe bei Hund und Katze und der Neugeborenenenerkrankungen hat das Werk deutlich gewonnen. Es gewinnt ebenfalls durch die Aktualisierung der wichtigsten Publikationen seit 1978, die den aktuellen Stand der klinikbezogenen Forschung widerspiegeln. Als modernes Lehrbuch werden mit folgenden Themen viele Informationen geboten: Geschichte, Bedeutung und Aufgaben der Tiergeburtsilfe in normaler Gravidität, Geburt und Puerperium, Endokrinologie der Hochträchtigkeit, der Geburt und des Puerperiums, die Milchdrüse während und nach der Geburt, Bedeutung des Herdengeschehens zum Geburtszeitpunkt sowie Pathologie der Geburt, des Puerperiums und der Nachkommen.

Als Standardwerk ist es jedem Studenten der Tiermedizin und jedem geburtsilflich tätigen Tierarzt zu empfehlen. Auch die Reduzierung des Preises auf 198,- DM sollte dabei ein weiterer Anreiz sein.

D. Rath, Mariensee

**A. Geißler, D. Schmitt, K.-F. Ziegahn: Standardisierung in der Umweltsimulation – insbesondere auf dem Gebiet der Luftreinhaltung.** Studie im Auftrag der Kommission Reinhaltung der Luft im VDI und DIN, Schriftenreihe der Kommission Reinhaltung der Luft im VDI und DIN, Bd. 20, 67 S., zahlr. Tab., Brosch., DM 24,- zuzüglich Versandkosten. Alleinvertrieb: Kommission Reinhaltung der Luft im VDI und DIN, Postfach 10 11 39, 40002 Düsseldorf.

Voraussagen über umweltbedingte Entwicklung bzw. die Abklärung von Interaktionen können durch Umweltsimulationen teilweise erkannt werden. Dies gewinnt nicht nur für technische Bereiche an Bedeutung, sondern findet auch im landwirtschaftlichen Umfeld seine Anwendung. Neben der Erfassung von Umwelteinflüssen müssen diese modellhaft nachgebildet werden, um die Wirkung beurteilen zu können und die Wechselwirkungen zu charakterisieren. Hierzu sind bestimmte Kenndaten erforderlich, von denen einige die interaktiven Prozesse beschreiben.

Die vorliegende Studie gibt einen Einblick in die Zusammenhänge zwischen Umweltsimulation und Reinhaltung der Luft und einen Überblick über die vorhandenen technischen Regeln wie DIN-Normen, Richtlinien, wichtige Gesetze und Verwaltungsvorschriften. Zum besseren Verständnis dieser Vernetzung werden die grundlegenden Aufgaben und Ziele sowie die Vorgehensweise der Umweltsimulation erläutert. Wenn es sich auch um ein rein technisches Buch handelt, wird es für denjenigen, der sich mit Tierhaltungssystemen beschäftigt, ein guter Leitfaden sein.

D. Rath, Mariensee

**Alois Essl: Statistische Methoden in der Tierproduktion.** Eine anwendungsorientierte Einführung. Österreichischer Agrarverlag, Wien. 316 S., 17 Abb., zahlr. Tabellen, brosch., DM 58,-. ISBN 3-7040-0859-1

Die Anwendung statistischer Verfahren ist in der Tierproduktion unerlässlich. Die rasante Entwicklung schneller Computersysteme mit hohen Rechnerkapazitäten erlaubt es, komplexe Rechenvorgänge kostengünstig und anwendungsbezogen durchzuführen. Oft fehlt aber ein ausreichendes statistisches Grundwissen. Das vorliegende Buch soll dem Rechnung tragen. Wie schon das Vorläuferbuch „Biometrische Methoden in der Tierproduktion“ von Haiger, 1966, richtet sich das neue Buch an alle in der Tierproduktion tätigen Wissenschaftler, Praktiker und Studenten.

Dem Leser werden zunächst nach einer kurzen Besprechung elementarer Begriffe und Maßzahlen vielfältige Anwendungsmöglichkeiten der Wahrscheinlichkeitsrechnung gezeigt. Zwei Kapitel über allgemeine Gesichtspunkte beim Testen von Hypothesen und Schätzen von Populationsparametern behandeln die grundlegenden Konzepte der analytischen Statistik. Eine kurze Einführung in das Rechnen mit Matrizen erleichtert den Zugang zu den heute allgemein verwendeten Test- und Schätzverfahren, die auf lineare Modellansätze zurückgehen. Hier wird neben der Regressions- und Korrelationsrechnung insbesondere auf das Schätzen von „fixen“ Effekten bei mehrfaktorieller Fragestellung sowie auf die Problematik der Zuchtwertschätzung eingegangen. Die beiden letzten Kapitel befassen sich mit nichtparametrischen Auswertungsmethoden und statistischen Aspekten in der Versuchspraxis.

Das Buch sollte an keinem Arbeitsplatz fehlen, wenn eine statistisch exakte Versuchsplanung und -bewertung erforderlich ist.

D. Rath, Mariensee

**Dedie, K., J. Bockemühl, H. Kühn, K.-J. Volkmer, T. Weinke: Bakterielle Zoonosen bei Tier und Mensch.** Mit einem Beitrag von H. Moegle. Ferdinand Enke Verlag 1993. XIV, 437 S., 124 Einzelabb., 43 Tab., geb., DM 198,-

Zoonosen sind nach WHO-Definition Infektionskrankheiten, die in natürlicher Weise zwischen Menschen und Wirbeltieren übertragen werden. Das vorliegende Buch behandelt ausschließlich bakterielle Zoonosen. Wichtige Saprozoonosen und Metazoonosen wie die Listeriose bzw. die Lyme-Borreliose werden ebenfalls berücksichtigt. Das Buch gliedert sich in 25 Kapitel. Einem Beitrag aus der allgemeinen Infektions- und Seuchenlehre, der auf die Thematik des Buches insofern eingeht, als auch grundlegende Definitionen (Zoonose, Saprozoonose, Metazoonose) aufgelistet werden, folgt die Darstellung von 24 bakteriellen Zoonosen in alphabetischer Reihenfolge. Jede Zoonose wird nach einem übersichtlichen Schema, das auch die schnelle Orientierung im Text erleichtert, abgehandelt. Störend wirkt sich an manchen Stellen das Bemühen des Verfassers aus, Krankheitsbezeichnungen abzukürzen, was dann zu

so unglücklichen Konstruktionen wie „Kkk“ für „Katzenkratzkrankheit“ führt. Im Kapitel über die Lyme-Borreliose gehen die Verfasser in dieser Hinsicht dann ganz eigene Wege. Anlässlich des 2. Internationalen Symposiums über die Lyme-Krankheit und verwandte Erkrankungen in Wien im Jahre 1985 wird international einheitlich die Krankheitsbezeichnung „Lyme-Borreliose“ verwendet. Im vorliegenden Buch muß sich der Leser erst an eine eher seltene Bezeichnung, nämlich „Schildzecken-Borreliose“, abgekürzt als „Se-Be“ oder „S-Be“ gewöhnen. Der Inhalt des Buches ist Gegenstand human- und veterinärmedizinischer Handbücher. Es ist jedoch auf jeden Fall wünschenswert, daß dieses weit verstreute Wissen gleichsam in einem Nachschlagewerk mit kurzen fundierten Darstellungen des aktuellen Kenntnisstandes der Zoonosenforschung zusammengefaßt wird. Das Buch wendet sich in erster Linie an praktizierende Ärzte und Tierärzte, darüber hinaus aber auch an Mikrobiologen und Pathologen. Allerdings muß man fragen, ob das Buch durch den Verzicht auf Berücksichtigung aktueller Entwicklungen, etwa im Bereich der diagnostischen Techniken, den Ansprüchen dieses Leserkreises gerecht werden kann.

In den Darstellungen zu einzelnen Zoonosen finden sich mißverständliche oder auch unrichtige Darstellungen. So ist es eine unzulässige Vereinfachung, die Beschreibung der Antigenstruktur von *Borrelia burgdorferi*, dem Erreger der Lyme-Borreliose, darauf zu beschränken, daß diese Bakterienspezies „zwei Oberflächenproteine aufweist“. Bis heute konnten hier etwa 40 verschiedene Oberflächenproteine nachgewiesen werden. Ein Beispiel mißverständlicher Darstellung, das deshalb so verwundert, weil einer der Verfasser vielbeachtete Arbeiten zu der angesprochenen Thematik veröffentlicht hat soll noch angeführt werden: „Veterinärmedizinisch hat *Campylobacter fetus* (...) seit etwa 1949 mit Einführen der technischen Besamung bei Rindern als Erreger von Fruchtbarkeitsstörungen erhebliche Bedeutung erlangt.“ Eigentlich ist es aber so, daß dieser wichtige Deckseuchenerreger durch die Ausweitung der künstlichen Besamung mit Sperma von seuchenhygienisch einwandfreien Besamungsbullen hierzulande weitgehend getilgt werden konnte. Weiterhin sind es Unterlassungen wie die unvollständige Wiedergabe von Rezepturen für Nährmedien sowie Druckfehler oder auch die Auswahl wenig aussagekräftiger Graphiken oder Photographien, über die man sich angesichts der Zusammensetzung des Autorenkollegiums, dessen fachliche Kompetenz doch allgemein anerkannt ist, verwundern muß. Andererseits bleibt anzuerkennen, daß kurze, informative und an vielen Stellen einprägsam bebilderte Abhandlungen zu einem derart wichtigen Thema wie den bakteriellen Zoonosen eine wertvolle Hilfe für die Arbeit von praktizierenden Ärzten und Tierärzten sein können.

Wittenbrink, Hannover

**Gotthard Ilchmann: Fachwörterbuch Veterinärmedizin.** Englisch-Deutsch-Französisch-Russisch mit ca. 13.000 Wortstellen. 1. Auflage, Verlag Alexandre Hatier, Berlin-Paris, 1993, 416 S., DM 198,- ISBN 3-86117-037-X

Die Anfertigung international anerkannter Publikationen, die Teilnahme an internationalen Kongressen sowie die multinationale Zusammenarbeit in Forschung, Lehre und bei landesübergreifenden praktischen tierärztlichen Aufgaben erfordern einen permanenten Informationsaustausch, bei dem es auf fachspezifische Termini ankommt und deren richtige Übersetzung Voraussetzung ist. Dem können Standardwörterbücher nicht gerecht werden. Nachdem für andere Disziplinen bereits Fachwörterbücher erschienen sind, haben die Autoren in dem vorliegenden Wörterbuch Fachbegriffe der Veterinärmedizin in deutscher, englischer, französischer und russischer Sprache zusammengestellt. Durch Indexzahlen lassen sich die 13.000 Begriffe in den verschiedenen Sprachen sehr schnell auffinden. Sie umfassen die Gebiete Morphologie, Physiologie, Biochemie, Tierverhalten, Pathologie, klinische Veterinärmedizin, Bakteriologie, Virologie, Bienenkrankheiten, Veterinärhygiene, Epidemiologie, Parasitologie, Pharmakologie, Toxikologie, veterinärmedizinische Aspekte der Tierhaltung, Tierzucht, Tierernährung und Futtermittelkunde sowie Fleischuntersuchung und Lebensmittelüberwachung.

Das Fachwörterbuch sollte auf keinem Arbeitsplatz fehlen, wenn internationale Kontakte zum täglichen Arbeitsbereich gehören.

D. Rath, Mariensee

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**Publishing and printing:** © 1994 Blackwell Wissenschafts-Verlag GmbH, Kurfürstendamm 57, D-10707 Berlin. Phone (+4930) 327 906-35. Fax: (+4930) 327 906-10. Printed in the UK by Page Bros, Norwich.

**Anzeigenverwaltung:** "Reproduction in Domestic Animals/Zuchthygiene", Blackwell Wissenschafts-Verlag GmbH, Kurfürstendamm 57, D-10707 Berlin. Phone (+4930) 327 906-29. d. Verantwortliche Anzeigenleiter: Leo Korff. Z. Zt. gilt die Anzeigenliste Nr. 13.

This journal is covered by Biosciences Information Service of Biological Abstracts and Current Contents (Series Agriculture, Biology & Environmental Sciences) of Institute for Scientific Information and Index Veterinarius and the Veterinary Bulletin of the Commonwealth Bureau of Animal Health.

ISSN 0936-6768

Hans G. Niemand / Peter F. Suter

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