EFFECTEN VAN EENVOUDIGE KOOLHYDRATEN EN FERMENTEERBARE KOOLHYDRATEN OP DE BINNEN-TOOM VARIATIE BIJ VARKENS

EINDRAPPORT VOOR HET PRODUCTSCHAP DIERVOEDER

Anne Wientjes, Nicoline Soede, Henry van den Brand, Bas Kemp

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Department of Animal Sciences, Wageningen University
P.O. Box 338
6700 AH Wageningen, The Netherlands

Deze eindrapportage bestaat uit de volgende onderdelen:

1. NEDERLANDSE SAMENVATTING EXPERIMENT 1 (FASE 1)

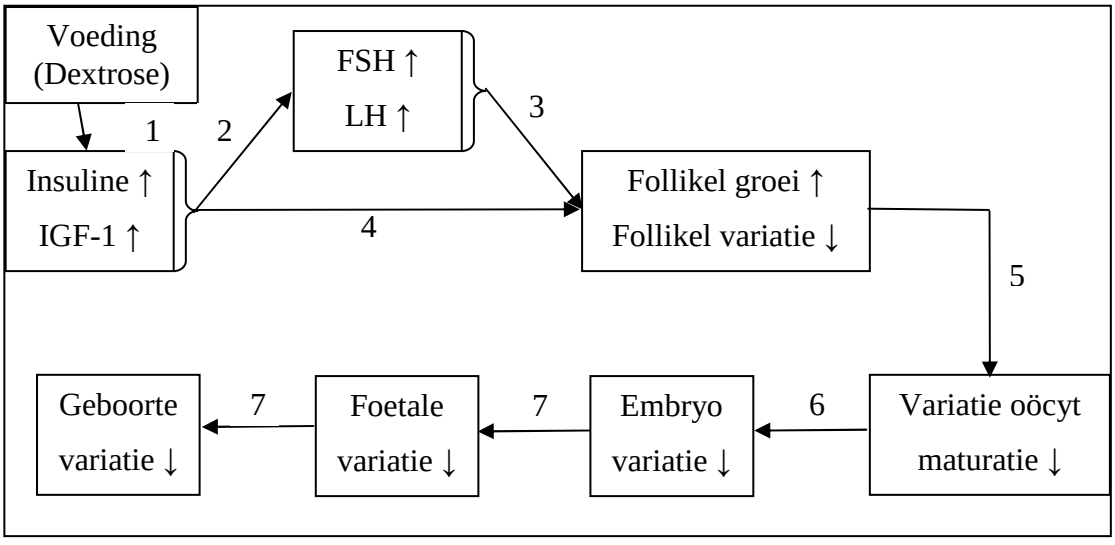
Achtergrond van de proef

In het ingediende projectvoorstel (VWV 07-52) is uitgebreid beschreven wat de achtergrond van het project is en wat de verwachting is t.a.v. het mechanisme dat ten grondslag ligt aan de relatie tussen de kwaliteit van follikelontwikkeling in de laatste dagen voor inseminatie, de ontwikkeling van embryo's en foeten tijdens de dracht en vervolgens de (variatie in) biggewicht van de tomen. Hieronder wordt de belangrijkste informatie herhaald.

Vermindering van variatie in geboortegewicht binnen een toom lijkt een goede optie om sterfte tijdens de lactatie en variatie in speengewicht te verminderen. De vraag is echter hoe dit bereikt kan worden. Van den Brand et al. (2006) lieten zien dat het voeren van dextrose (150 gram per dag) tijdens het interval spenen-bronst een significante vermindering (P=0.03) van de binnen-toom variatie in geboortegewicht opleverde. Daarnaast was het percentage biggen <1000 g numeriek lager (5.1 vs 8.1%, P=0.17) in de dextrose gevoerde zeugen. In een volgend experiment (Van den Brand et al., 2009) werden in een enigszins gewijzigde proefopzet (dextrose plus lactose gevoerd vanaf moment van opleg in de kraamstal (ongeveer dag 110) tot en met einde bronst) vergelijkbare resultaten gevonden op de binnen-toom variatie in geboortegewicht en ook waren deze biggen gemiddeld 84 gram zwaarder (1483 vs 1569; P=0.05). In Engeland voerden Ferguson et al. (2006) vezelrijke voeders in de cyclus voor inseminatie en vonden een lagere variatie in foetaal gewicht op dag 27 van de dracht. Vezelrijke voeders hebben, net als dextrose, een insuline stimulerende werking. Uit deze en andere onderzoeken concluderen we dat de voeding in de periode voor inseminatie (in ieder geval de folliculaire fase, en wellicht ook eerder, tijdens lactatie) effect heeft op de binnen-toom variatie in geboortegewicht, terwijl er aanwijzingen zijn dat daardoor ook inderdaad de sterfte van (met name lichte) biggen afneemt.

Mogelijk mechanisme

Het mogelijke mechanisme van dextrose, verstrekt tijdens de folliculaire fase, op de binnen-toom variatie in geboortegewicht in de volgende worp is in figuur 1 weergegeven. In dit figuur en de uitleg wordt uitgegaan van dextrose, maar van lactose wordt een vergelijkbaar effect verwacht.



Figuur 1. *Mogelijk mechanisme betreffende de relatie tussen dextrose, verstrekt tijdens de folliculaire fase, en de binnen-toom variatie in geboortegewicht.*

1. Effect van dextrose op insuline en IGF-1

Dextrose in het rantsoen van zowel gelten als lacterende zeugen gaf een snelle, meer langdurige, insuline piek na het voeren (Van den Brand et al., 1998; 2000; Ziecik et al., 2002). Ook werd in de dextrose-zeugen tijdens/na lactatie het IGF-1 gehalte verhoogd (Van den Brand et al., 2001).

2. Relatie insuline/IGF-1 en FSH/LH

In studies waarin elk uur werd gevoerd werd relatie gevonden tussen insuline en LH (Tokach et al., 1992; Koketsu et al., 1996). In studies waarin 2x per dag werd gevoerd werd geen relatie gevonden (Paterson and Pearce, 1994; Quesnel et al., 1998; Van den Brand et al., 2000). Een langdurig verhoogd insuline niveau lijkt dus van groter belang dan een hoge insuline piek. IGF-1 niveaus zijn veel constanter gedurende de dag en de positieve relatie tussen IGF-1 en LH is duidelijk en consistent (Tokach et al., 1993; Van den Brand, 2000). Er is niets bekend over een relatie tussen insuline/IGF-1 en FSH. Echter, er zijn insuline receptoren in de hypothalamus en hypofyse (Booth, 1990), dus zou je verwachten dat LH en FSH beide worden gestimuleerd. En bovendien verloopt natuurlijk stimulatie van zowel LH als FSH via het hypothalame GnRH, dus is het aannemelijk dat, wanneeer LH wordt gestimuleerd, ook FSH wordt gestimuleerd.

3. en 4. Relatie insuline/IGF-1 of FSH/LH en folliculaire ontwikkeling

Zoals eerder beschreven onder 2, stimuleren insuline en IGF-1 de productie van LH en mogelijk FSH op hypofyse niveau, wat kan zorgen voor een betere stimulering van de groei van de follikels, met name follikels die relatief nog klein zijn en weinig LH receptoren hebben. Het idee is nu dat insuline FSH stimuleert, waardoor minder follikels in atresie gaan en follikels die niet in atresie gaan, worden gestimuleerd, zodat ze gevoeliger worden voor LH. Daarnaast heeft insuline een direct effect op follikels; toename van LH receptoren op de granulosacellen (Poretsky en Kalin, 1987), aromatase activiteit en synthese van steroidhormonen (Matamoros et al., 1990, 1991). Door dit alles neemt, naar verwachting, de homogeniteit in follikelontwikkeling toe.

5. Relatie tussen folliculaire ontwikkeling en eicelkwaliteit

Zak et al. (1997) lieten zien dat zeugen die tijdens de laatste week van lactatie beperkt gevoerd werden, zowel het aantal grote follikels kleiner was, en ook de ontwikkelingspotentie van de eicellen in deze follikels verminderd was; ook de follikelvloeistof bleek minder goed in staat om eicellen tot ontwikkeling te brengen. De conclusie was dat een minder goede follikelontwikkeling ook consequenties heeft voor de ontwikkeling van de eicellen. Xie et al. (1987) lieten zien dat er zowel tussen zeugen als binnen zeugen een forse variatie is in eicelontwikkeling. De hypothese is nu dat als gevolg van een stijging van insuline en/of IGF-1 de follikelgroei en eicelkwaliteit gestimuleerd wordt en deze meer homogeen wordt.

6. De relatie tussen follikel/eicel kwaliteit en embryokwaliteit

Pope et al. (1990) en Xie et al. (1990) lieten zien dat uit follikels die als eersten ovuleerden (de verder ontwikkelde follikels) ook verder ontwikkelde embryo's kwamen, ondanks dat het ovulatieproces relatief weinig tijd in beslag neemt (Soede et al., 1992; Kemp en Soede, 1993).

7. Relatie vroeg embryonale variatie en geboortegewicht variatie

Op dag 13 tot 14 na de bevruchting vindt de implantatie plaats. Wanneer er een grote variatie is in embryonale ontwikkeling voor dag 13, zullen tijdens de implantatie de verder ontwikkelde embryo's meer ruimte in beslag nemen dan de minder ver ontwikkelde embryos. Dat kan resulteren in afsterven van de kleine embryo's in de verdere ontwikkeling (Pope and First, 1985; Wilmut et al., 1985), dan wel in het achterblijven in groei van deze embryo's, waardoor de variatie aan het einde van de embryonale fase (dag 30-35) en bij de geboorte groter wordt. Van der Lende et al. (1990) lieten zien dat de variatie in ontwikkeling binnen een zeug aan het einde van de embryonale fase zeer representatief is voor de variatie in geboortegewicht binnen een zeug.

Samenvattend, stimulering van de insuline en/of IGF-1 afgifte voorafgaand aan en tijdens de folliculaire fase heeft mogelijk een positief effect op de variatie in antrale follikel pool met als gevolg minder variatie in: de pre-ovulatoire follikel pool, de oöcyt ontwikkeling, de embryo-ontwikkeling aan het einde van de embryonale fase, en uiteindelijk het geboortegewicht binnen de toom.

Doel van het onderzoek
Het doel van dit onderzoek was nader inzicht te krijgen in het mechanisme dat ten grondslag ligt aan de relatie tussen insuline-stimulerend voer (dextrose+lactose) tijdens het interval spenen-ovulatie, follikelontwikkeling in de laatste dagen voor inseminatie en (variatie in) ontwikkeling van embryo's tijdens de vroege dracht.

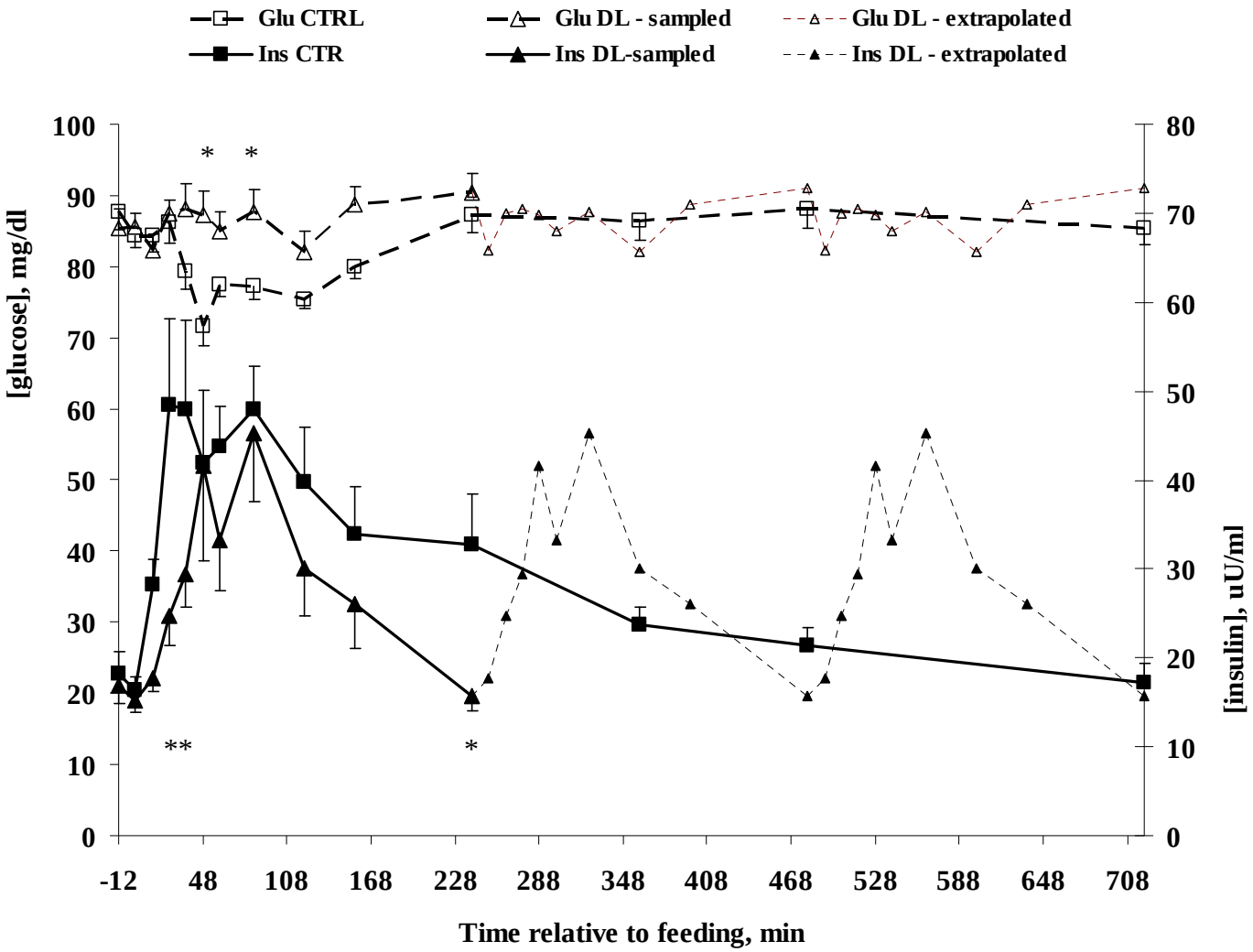
Proefopzet
Multipare Topigs 20 zeugen (n=49) kregen tijdens het interval spenen-ovulatie ofwel een controlevoer (**CTRL**), ofwel een insuline-stimulerend voer (**DL**). Deze bestonden uit hetzelfde basisvoer (3.5kg), waarin ofwel soja olie (CTRL; dagelijks 108 g), ofwel dextrose+lactose (DL; dagelijks 150g + 150 g) was bijgemengd. Alle dieren ontvingen dezelfde hoeveelheid energie (iso-calorisch) en eiwit. In de behandelingsgroep (DL) werd het voer in 6 voerbeurten per dag verstrekt (elke 4 uur) en de controle (CTRL) zeugen werden 2 keer per dag gevoerd (8.00u en 20.00u). Dit werd gedaan om het insulineniveau in het bloed zo hoog mogelijk, maar ook zo constant mogelijk te houden in de DL groep, en tevens om een duidelijk contrast te creëren tussen beide behandelingen. Vanaf ovulatie kregen alle zeugen 3.0 kg van het basisvoer, evenredig verdeeld over 2 voerbeurten per dag (8.00u en 20.00u). 30 zeugen kregen op de dag van spenen een vene jugularis canule om gedurende de hele periode (vanaf spenen tot aan slacht) hormoonprofielen (LH, P4) in het bloed te kunnen bepalen; de overige 20 zeugen kregen een oorcanule op de dag van spenen tot aan ovulatie, om hormoonprofielen tijdens het interval spenen-ovulatie (LH) te bepalen. Tevens zijn op dag 2 en 3 na spenen van alle zeugen glucose- en insulineprofielen rondom voerbeurten bepaald (in DL t/m 4u na voeren, omdat zeugen daarna weer gevoerd werden; in CTRL t/m 12u na voeren) en werd dagelijks een bloedmonster genomen voor de bepaling van IGF-1. Op dag 10/11 van de dracht zijn de zeugen geslacht, baarmoederhoorns gespoeld en embryo’s en ovaria beoordeeld.

Zieke dieren
Een aantal zeugen is ziek geworden tijdens het onderzoek, waaronder alle 11 zeugen uit batch 4. De gegevens van de veterinair behandelde/zeugen met koorts zijn nader geanalyseerd om te kijken of koorts/behandeling de insuline niveaus, IGF-1 niveaus en embryonale overleving beïnvloed heeft. Hiervoor is een indeling gemaakt in 1). Gezond: dieren met gedurende de hele proef een lichaamstemperatuur <38,9ºC en geen stijgingen van >0,5 ºC, en niet veterinair behandeld, 32 dieren (15 CTRL en 17 DL); 2). Ziek/behandeld: dieren met een of enkele dagen een lichaamstemperatuur >38,9ºC en veterinair behandeld, 17 dieren (9 CTRL en 8 DL). Uit de analyses bleek dat ziekte/koorts zowel de insuline en IGF-1 niveaus, als het aantal embryo’s en embryonale overleving negatief beïnvloed had. Daarom is besloten om voor de uiteindelijke analyses alleen de gezonde dieren mee te nemen (n=32 zeugen).

Resultaten
Resultaten zijn uitgedrukt als gemiddelden±SE, tenzij anders vermeld. Voor een compleet overzicht van de analyses en resultaten, wordt verwezen naar de twee artikelen betreffende dit experiment (bijlage B en C).

Voeropname
Er was een grote spreiding in voeropname tijdens het interval spenen-ovulatie tussen individuele zeugen, maar de voeropname verschilde niet tussen de behandelingen (respectievelijk 63±10% en 73±6% in CTRL en DL; P=0,37). Na ovulatie hadden alle zeugen een 100% voeropname.

Glucose, insuline en IGF-1
Glucose en insuline profielen van de zeugen die een goede voeropname (≥75% droge stof opname) hadden op de dagen waarop glucose en insuline profielen bepaald zijn (d2 en d3 na spenen) zijn weergegeven in figuur 2.



Figuur 2. *Glucose en insuline profielen (means±SE) rondom de 8u voerbeurt voor zeugen met een droge stof opname ≥75% op d2 en d3 na spenen; DL = 18 zeugdagen, glucose- en insuline niveaus bepaald van -12 t/m 240 min na voeren, daarna geëxtrapoleerd; CTRL = 16 zeugdagen, glucose- en insuline niveaus bepaald van -12 t/m 720 min na voeren;* * *= CTRL vs. DL, P≤0,05*

Basaal glucose concentratie was vergelijkbaar voor beide behandelingen, maar glucose oppervlakte onder de curve (AUC) was lager in CTRL dieren dan in DL dieren (AUC/4u was -1.382±365 mg in CTRL en 317±345 mg in DL, P=0,0001; AUC/12u was -946±1.362 mg in CTRL en 950±1.034 mg in DL, P=0,07).

Insuline AUC/4u was hoger in CTRL dieren dan in DL dieren (5.021±676 µU in CTRL en 2.856±374 µU in DL, P=0,03), maar basaal insuline, insuline piekhoogte (absoluut en relatief), AUC/12u en gemiddeld insuline niveau verschilde niet tussen de behandelingen.
IGF-1 niveaus tijdens het interval spenen-ovulatie lieten een stijging zien vanaf d1 tot d3 na spenen, maar de niveaus verschilden niet tussen behandelingen. Wel waren IGF-1 niveaus hoger in zeugen met een goede voeropname (≥75% tijdens het interval spenen-ovulatie) op d4 en d5 na spenen (LSmeans waren respectievelijk 152,5 en 174,9 ng/ml op d4 (P=0,01) en 143,6 en 169,9 ng/ml op d5 (P=0,001) voor zeugen met een lage en hoge voeropname).

Follikel ontwikkeling, bronst en ovulatie
De gemiddelde diameter van de 5 grootste follikels op de dag van spenen, d4 na spenen en bij ovulatie, het interval spenen-ovulatie, bronstduur, ovulatiegraad, basaal LH niveau rondom de LH piek en het interval spenen-LH piek verschilden niet tussen behandelingen, en waren niet beïnvloed door voeropname tijdens het interval spenen-ovulatie.
De pre-ovulatoire LH piek was hoger in de CTRL dieren (Tabel 1), en was hoger in dieren met een lage (<75%) voeropname vergeleken met dieren met een hoge (≥75%) voeropname tijdens het interval spenen-ovulatie (LSmeans waren respectievelijk 3,6 en 3,1 ng/ml voor zeugen met een lage en hoge voeropname; P=0,06).
Het aantal follikels in de gehele follikelpool op d4 na spenen verschilde niet tussen behandelingen, maar de gemiddelde diameter en de uniformiteit van deze follikels (SD en CV) waren hoger in CTRL dieren dan in DL dieren (Tabel 2).

Tabel 1. *LH, luteale ontwikkeling en progesteron kenmerken die beïnvloed zijn door de behandeling en/of voeropname tijdens het interval spenen-ovulatie (means±SE)*

	CTRL (n=15)	**DL (n=16)[3]**	**P-waarde[1] Behandeling**	**Voeropname iso[2]**
LH piekhoogte, ng/ml	3,73±0,24	3,00±0,18	0,03	0,06[6]
Totaal luteaal gewicht, g[4]	11,2±0,5	9,7±0,5	0,03	n.s.
CL diameter, mm[4]	10,0±0,3	9,6±0,3	0,06	<0,01[6]
CL gewicht, g[4]	0,47±0,02	0,42±0,02	0,09	n.s.
Basaal progesteron, ng/ml[5]	0,49±0,07	0,86±0,21	0,23	0,06[6]
Gemiddeld progesteron, ng/ml[5]	14,60±1,35	14,70±0,90	0,96	0,05[6]

[1] Statistische significantie; de behandeling*voeropname-interactie was niet significant (P>0,10); [2] Voeropname vanaf spenen tot aan 12u na ovulatie, in % droge stof opname (<75%, ≥75%); [3] 1 DL zeug ontwikkelde cysteuze ovaria; niet meegenomen in de analyses; [4] 2 CTRL zeugen ontwikkelde cysteuze corpora lutea; niet meegenomen in analyses; [5] Alleen voor zeugen met een vene jugularis-canule (10 CTRL en 11 DL zeugen); [6] Voor zeugen met lage (<75%) en hoge (≥75%) voeropname, LSmeans waren respectievelijk 3,6 and 3,1 ng/ml voor LH piekhoogte, 10,4 en 9,4 mm voor CL diameter, 0,89 en 0,45 ng/ml voor basaal progesteron, en 13,60 en 16,31 voor gemiddeld progesteron.

Luteale ontwikkeling en progesteron
Totaal luteaal gewicht, gemiddelde corpus luteum diameter en gemiddeld gewicht van de corpora lutea (Tabel 1) waren hoger in CTRL dieren dan in DL dieren. Daarnaast hadden zeugen met een lage (<75%) voeropname tijdens het interval spenen-ovulatie grotere corpora lutea dan zeugen met een hoge (≥75%) voeropname (LSmeans waren respectievelijk 10,4 en 9,4 mm voor zeugen met een lage en hoge voeropname; P<0,01).
Progesteron niveaus in plasma (basaal, gemiddeld en maximaal) tijdens de vroege dracht waren vergelijkbaar voor beide behandelingen. Basaal progesteron (gemiddelde progesteron niveau tussen 6 en 2u vóór ovulatie) tendeerde naar een hoger niveau in dieren met een lage voeropname

tijdens het interval spenen-ovulatie (LSmeans waren respectievelijk 0,89 en 0,45 ng/ml voor zeugen met een lage (<75%) en hoge (≥75%) voeropname; P=0,06), terwijl gemiddeld progesteron niveau tijdens de eerste 10d van de dracht hoger was in dieren met een hoge voeropname tijdens het interval spenen-ovulatie (LSmeans waren respectievelijk 13,60 en 16,31 ng/ml voor zeugen met een lage (<75%) en hoge (≥75%) voeropname; P=0,05).

Corpus luteum diameter en gewicht en totaal luteaal gewicht waren onderling hoog gecorreleerd (r≥0,45; P<0,01). Corpus luteum-ontwikkeling (diameter, gewicht en totaal gewicht) was niet gecorreleerd met gemiddeld progesteron niveau tijdens de vroege dracht; totaal luteaal gewicht was wel sterk gecorreleerd met maximaal progesteron niveau op d10 van de dracht (r=0,51; P=0,005).

Tabel 2. *Follikel pool kenmerken op d4 na spenen (means±SE)*

	CTRL (n=9)[2]	**DL (n=11)[2]**	**P-waarde behandeling[1]**
Aantal follikels	21,1±0,8	22,1±0,7	0,39
Gemiddelde diameter, mm	6,5±0,2	6,1±0,1	0,08
SD, mm	0,72±0,04	0,88±0,06	0,05
CV, %	11±1	15±1	0,02

[1] Statistische significantie; het effect van voeropname en de behandeling*voeropname-interactie waren niet significant (P>0,10); [2] 1 DL zeug ontwikkelde cysteuze ovaria en is daarom niet meegenomen in de analyses; voor 11 zeugen (6 CTRL en 5 DL) waren geen complete scanfilmpjes van beide eierstokken beschikbaar.

Om erachter te komen of de betere luteale ontwikkeling (corpus luteum diameter en gewicht en totaal luteaal gewicht) in de CTRL dieren gerelateerd is aan de betere follikelontwikkeling in deze dieren, zijn correlaties berekend tussen follikelontwikkeling parameters en corpora lutea parameters, en zijn follikelparameters als verklarende variabelen toegevoegd aan de modellen voor luteale ontwikkeling. De gemiddelde diameter van de 5 grootste follikels op d4 na spenen en bij ovulatie waren niet gecorreleerd met corpus luteum diameter of gewicht. De gemiddelde diameter van de gehele follikelpool op d4 na spenen (bepaald voor de 20 zeugen met complete scanfilmpjes van beide eierstokken) was sterk gecorreleerd met zowel corpus luteum diameter (r=0,55; P=0,02), corpus luteum gewicht (r=0,61; P=0,007), als totaal luteaal gewicht (r=0,54; P=0,02). Toevoeging van gemiddelde follikeldiameter op d4 aan het model voor zowel corpus luteum diameter als corpus luteum gewicht bevestigde de hypothese dat de betere corpus luteum ontwikkeling in de CTRL dieren (deels) verklaard kan worden door de betere follikelontwikkeling in deze zeugen (P-waardes voor het effect van gemiddelde follikeldiameter waren 0,03 (ß=1,23 mm / mm) en 0,07 (ß=0,06 g / mm) voor respectievelijk CL diameter en CL gewicht).

Embryo ontwikkeling en uniformiteit

In totaal waren 28 van de 32 zeugen drachtig (14 CTRL en 14 DL; 1 DL zeug had een stille bronst, 1 DL zeug ontwikkelde cysteuze ovaria, en 2 zeugen (1 CTRL en 1 DL) hadden een baarmoederontsteking, en geen vitale embryo's).

Het aantal embryo's en de embryonale overleving waren vergelijkbaar tussen behandelingen en niet beïnvloed door voeropname tijdens het interval spenen-ovulatie. Embryo ontwikkeling (diameter, oppervlak, embryoblast diameter, eiwit inhoud en DNA inhoud) en uniformiteit (SD en CV van bovengenoemde kenmerken) waren niet beïnvloed door voeropname tijdens het interval spenen-ovulatie. Embryo diameter tendeerde naar een hoger niveau in CTRL dieren (Tabel 3); embryo oppervlak, embryoblast diameter, eiwit inhoud en DNA inhoud waren alleen numeriek hoger in de CTRL dieren (Tabel 3).

Uniformiteit van de embryo's (SD en CV van alle embryo kenmerken) verschilde niet tussen behandelingen.

Tabel 3. *Embryo-ontwikkeling op d10 van de dracht (means±SE)*

	CTRL (n=13)[2]	**DL (n=12)[2]**	**P-waarde behandeling[1]**
Embryo diameter, mm	7,1±0,47	6,4±0,64	0,07
Embryo oppervlak, mm^2	73,0±10,5	63,4±11,2	0,11
Embryoblast diameter, mm	0,43±0,02	0,40±0,03	0,20
Eiwit inhoud, µg	86±9	75±11	0,29
DNA inhoud, ng	349±33	329±36	0,32

[1] Statistische significantie; het effect van voeropname en de behandeling*voeropname-interactie waren niet significant (P>0,10), wel is gecorrigeerd voor de leeftijd van de embryo's (9,5 vs. 10d); [2] 5 zeugen waren niet drachtig, en daarnaast hadden 2 zeugen al filamenteuze embryo's, waardoor het niet mogelijk was de ontwikkeling van de individuele embryo's te bepalen.

Relaties tussen insuline en IGF-1 niveaus en reproductiekenmerken
Om relaties te bepalen tussen insuline parameters en IGF-1 niveaus tijdens het interval spenen-ovulatie met de verschillende reproductiekenmerken, zijn de volgende insuline en IGF-1 parameters gebruikt:

- basaal insuline (gemiddelde waarde van d2 en d3 per zeug)
- insuline AUC/12u (gemiddelde waarde van d2 en d3 per zeug)
- gemiddeld insuline niveau (gemiddelde waarde van d2 en d3 per zeug)
- gemiddeld IGF-1 niveau gedurende d3-5 na spenen

De correlaties tussen deze verschillende insuline en IGF-1 parameters waren als volgt: basaal insuline en insuline AUC/12u waren niet gecorreleerd (P=0,22), maar gemiddeld insuline was hoog gecorreleerd met zowel basaal insuline (r=0,69; P<0,0001) als insuline AUC/12u (r=0,86; P<0,0001); gemiddelde IGF-1 niveau gedurende d3-5 na spenen was gecorreleerd met basaal insuline (r=0,39; P=0,03) en gemiddelde insuline (r=0,39; P=0,03), maar niet met insuline AUC/12u (P=0,17).
In de analyses bleek dat de interactie tussen behandeling en insuline/IGF-1 parameters nergens significant was (P>0,10). Daarom zijn overall regressies uitgevoerd (wel met correctie voor het behandelingseffect). De significante relaties die hieronder besproken worden zijn in figuren weergegeven in de bijlage.

Follikel ontwikkeling, bronst en ovulatie. Basaal insuline niveau was positief gerelateerd met follikel diameter bij ovulatie (ß=0,05 mm/(µU/ml); P=0,04; figuur 1A) en negatief gerelateerd met LH piekhoogte (ß=-0,07 (ng/ml)/(µU/ml); P=0,01; figuur 1B).
Een positieve relatie bestond tussen zowel insuline AUC/12h (ß=0,015 (ng/ml)/(1.000 µU); P=0,05; figuur 2A), gemiddeld insuline niveau (ß=0,007 (ng/ml)/(µU/ml); P=0,05; figuur 2B), en gemiddeld IGF-1 niveau gedurende d3-5 na spenen (ß=0,002 (ng/ml)/(ng/ml); P<0,01; figuur 2C) met basaal LH niveau.

Luteale ontwikkeling en progesteron. Gemiddeld insuline niveau liet een negatieve relatie zien met gemiddelde corpus luteum diameter (ß=-0,06 mm/(µU/ml); P=0,01; figuur 3). Zowel insuline AUC/12u (ß=0,35 (ng/ml)/(1.000 µU); P=0,02; figuur 4A) als gemiddeld insuline niveau (ß=0,14 (ng/ml)/(µU/ml); P=0,05; figuur 4B) waren positief gerelateerd met gemiddeld progesteron niveau gedurende de eerste 10d van de dracht, en met maximaal progesteron niveau op d10 van de dracht

(insuline AUC/12u: ß=0,73 (ng/ml)/(1.000 µU); P=0,0046; figuur 5A; gemiddelde insuline niveau: ß=0,27 (ng/ml)/(µU/ml); P=0,05; figuur 5B).
Embryo ontwikkeling. Insuline AUC/12u liet een positieve relatie zien met embryo diameter (ß=0,15 mm/(1.000 µU); P=0,03; figuur 6). Daarnaast waren er tendensen voor positieve relaties tussen insuline AUC/12u met embryo oppervlak (ß=2,46 mm^2/(1.000 µU); P=0,06) en embryo eiwit (ß=2,13 µg/(1.000 µU); P=0,08), en voor positieve relaties tussen gemiddeld insuline niveau met embryo diameter (ß=0,06 mm/(µU/ml); P=0,09).

Discussie
Insuline niveaus tijdens het interval spenen-ovulatie zijn gerelateerd met: *I). follikelontwikkeling* (basaal insuline was positief gerelateerd aan follikel diameter bij ovulatie); *II). LH afgifte* (basaal insuline was negatief gerelateerd met LH piekhoogte, maar zowel insuline AUC/12u en gemiddeld insuline niveau waren positief gerelateerd aan basaal LH niveau); *III). luteale ontwikkeling en progesteron* (gemiddeld insuline niveau was negatief gerelateerd aan corpus luteum diameter, maar zowel insuline AUC/12u als gemiddeld insuline niveau waren positief gerelateerd aan gemiddeld en maximaal progesteron niveau gedurende de vroege dracht); *en IV). embryo ontwikkeling* (insuline AUC/12u was positief gerelateerd aan embryo diameter). Dit suggereert dat hoge insuline niveaus tijdens het interval spenen-ovulatie leiden tot een beter uitgangspunt voor goede embryonale overleving en ontwikkeling (hoger progesteron en verder ontwikkelde embryo's).

Onze oorspronkelijke hypothese was dat insuline-stimulerende voeding tijdens het interval spenen-ovulatie leidt tot een uniformere pre-ovulatoire follikel pool (doordat met name de follikels die relatief nog klein zijn en weinig LH receptoren hebben, extra gestimuleerd worden), waardoor enerzijds uniformere embryo's ontstaan en anderzijds een betere progesteron productie door de corpora lutea. Het uiteindelijke resultaat zou dan een uniformere toom bij geboorte zijn.
De resultaten uit dit experiment bevestigen deze hypothese deels. Er bestaan positieve relaties tussen insuline niveaus en zowel follikel ontwikkeling als embryo ontwikkeling. De positieve relaties tussen follikel diameter en corpus luteum ontwikkeling (diameter en gewicht) bevestigen dat verbeterde follikelontwikkeling daarnaast resulteert in een betere luteale ontwikkeling. Bovendien was er inderdaad een positieve relatie tussen insuline niveaus tijdens het interval spenen-ovulatie en progesteron productie in de vroege dracht.
De uniformiteit van zowel follikels als embryo's leek echter niet beïnvloed door insuline. Dit zou kunnen betekenen dat insuline niet zozeer de uniformiteit van follikels en embryo's verhoogt, maar de algehele ontwikkeling van zowel follikels als embryo's stimuleert, en dat effecten op uniformiteit wellicht pas later zichtbaar worden. Hoe een betere follikel- en embryo-ontwikkeling vervolgens leidt tot uniformere tomen bij geboorte moet verder onderzocht worden (er zal immers nog een deel van de embryo's afsterven tijdens de verdere dracht). Een hypothese is dat insuline-stimulatie tijdens het interval spenen-ovulatie leidt tot een betere algehele embryonale ontwikkeling, maar daarnaast ook tot een betere embryonale overleving en placenta-ontwikkeling, wat gunstig zou kunnen zijn voor de uniformiteit van de biggen bij geboorte.

Hoge insuline niveaus tijdens het interval spenen-ovulatie lijken dus van belang voor een goede dracht. In dit experiment is getracht door middel van het frequent voeren (6x/dag) van een insuline-stimulerend voer (dextrose plus lactose) insuline niveaus zo hoog en constant mogelijk te houden voor een groot deel van de dag, maar dit had niet het verwachte resultaat; de insuline piekhoogte en totale insuline afgifte gedurende de dag verschilde niet tussen de behandelingen. Het was in dit experiment niet mogelijk de effecten van voersamenstelling en voerfrequentie (=portiegrootte) te scheiden, maar in een vervolg experiment (*experiment 2, wordt in hoofdstuk 2 verder toegelicht*) is dit wel onderzocht. De CTRL en DL voeders zijn daarbij gevoerd aan guste

zeugen in porties van 1,5kg (2x/dag), en hier resulteerde het DL voer in een hogere insuline respons vergeleken met het CTRL voer (AUC/6.2u was respectievelijk 3.801±326 vs. 2.842±310 μU voor het DL en CTRL voer). Dit geeft aan dat het insuline-stimulerende effect van het DL voer in het huidige experiment teniet gedaan werd door de kleinere porties (zie resultaten en discussie hoofdstuk 2).

De resultaten van dit experiment geven verder aan dat niet alleen de absolute afgifte van insuline van belang is voor stimulatie van follikels, corpora lutea en embryo's, maar ook dat het patroon waarin insuline wordt afgegeven een rol speelt. De CTRL dieren hadden namelijk grotere follikels op d4, hogere LH piekhoogtes, zwaardere en grotere corpora lutea en verder ontwikkelde embryo's op d10 van de dracht, ondanks gelijke totale insuline afgifte met de DL dieren. Dit suggereert dat tweemaal daags een grote insuline-stimulatie voordeliger is dan dezelfde hoeveelheid insuline afgegeven in meer frequente, maar kortere insuline-pieken. Kortom, 2x/dag voeren lijkt gunstiger voor follikelontwikkeling en dracht dan frequenter voeren.

Om de vraag te beantwoorden welke voeders de meeste potentie hebben om insuline afgifte bij zeugen te stimuleren, is een 2^{e} experiment uitgevoerd.

2. NEDERLANDSE SAMENVATTING EXPERIMENT 2

Doel van de proef
Het doel van de proef was om nader inzicht te krijgen in de directe effecten van de specifieke voercomponenten dextrose, lactose, sucrose en suikerbietenpulp (zowel apart als gecombineerd) op glucose, insuline en IGF-1 profielen in zeugen om zo diëten te vinden met de hoogste potentie om insuline en IGF-1 afgifte te stimuleren.

Proefopzet
14 guste oudere-worps Topigs 20 zeugen (hersteld van lactatie (>30d na spenen) en onder dagelijkse regumate behandeling om bronst en daarmee gepaard gaande voerresten te voorkomen), kregen verschillende proefvoeders gevoerd gedurende 6 achtereenvolgende proefperiodes. De volgende proefvoeders zijn getest:

1. Controle (CON); basisvoer met sojaolie (vergelijkbaar met controle voer uit exp. 1);
2. Dextrose (D); basisvoer inclusief dagelijks 150g dextrose;
3. Lactose (L); basisvoer inclusief dagelijks 150g lactose;
4. Dextrose plus lactose (DL); basisvoer inclusief dagelijks 150g dextrose plus 150g lactose;
5. Sucrose (S); basisvoer inclusief dagelijks 150g sucrose;
6. Sucrose plus lactose (SL); basisvoer inclusief dagelijks 150g sucrose en 150g lactose;
7. Dextrose/Suikerbietenpulp (DSBP); losstaand voer vergeleken met de rest (wel isocalorisch en isonitrogeen), met dagelijks 1200g suikerbietenpulp (t.o.v. dagelijks 360g suikerbietenpulp in CTRL voer) en 240g dextrose.

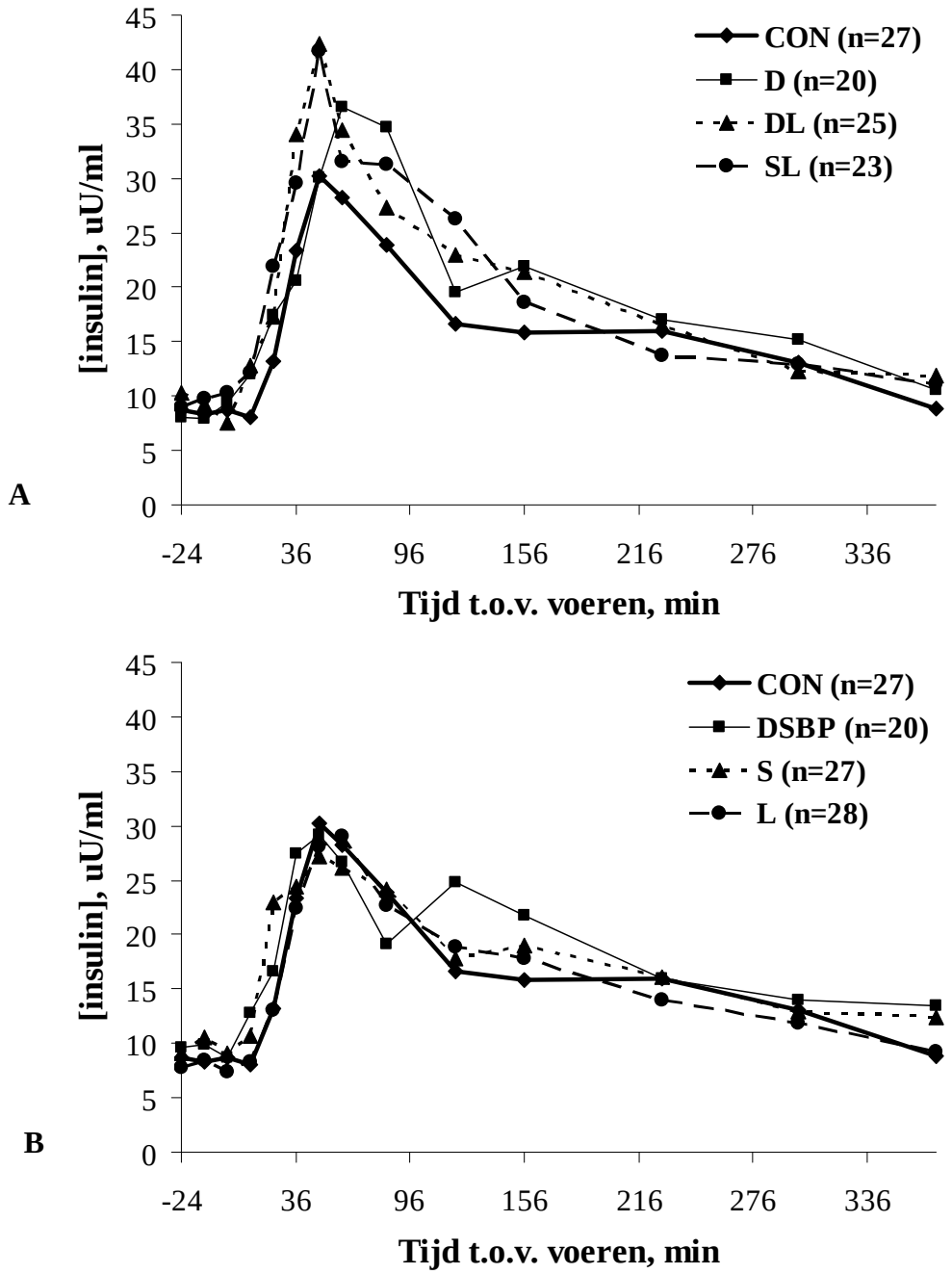
Het DL voer (dextrose plus lactose) komt overeen met het DL voer uit experiment 1, en om afzonderlijke effecten van zowel dextrose als lactose te onderzoeken zijn ook de voeders met ofwel dextrose (D) ofwel lactose (L) toegevoegd. In een onderzoek met biologische zeugen is gekeken naar het effect van sucrose plus lactose (SL) op biguniformiteit (omdat geen biologische dextrose beschikbaar is; Van der Peet-Schwering & Binnendijk, in voorbereiding), vandaar dat in dit experiment ook gekeken wordt naar de specifieke effecten van sucrose (S) en de combinatie van sucrose plus lactose (SL) op insuline afgifte. Tenslotte toonde Vestergaard (1997) aan dat suikerbietenpulp zorgt voor een langdurige verhoging van insuline afgifte na het voeren, wat zou kunnen betekenen dat de combinatie dextrose plus suikerbietenpulp (DSBP) wellicht tot de hoogste insuline afgifte zou kunnen leiden.
De voeders 1 t/m 6 bestonden uit hetzelfde basisvoer, waarin de additionele voercomponenten waren bijgemengd. Sojaolie is gebruikt om alle voeders isocalorisch te maken. Daarnaast waren alle voeders isonitrogeen.
Er werd gebruik gemaakt van een latijns vierkant, dwz elke zeug kreeg achtereenvolgens de verschillende proefvoeders en was zo haar eigen controle. Elk proefvoeder werd gedurende een periode van 9,5 achtereenvolgende dagen gevoerd (om te zien of een adaptatieperiode nodig was voor bijv. de opbouw van de juiste darmflora), in 2 gelijke porties per dag (3kg/dag). Na elke proefperiode kregen alle dieren gedurende 4,5d het basisvoer (op individueel onderhoudsniveau), om zo een rustperiode in te bouwen en carry-over effecten van verschillende voeders te minimaliseren.
Op d2, d5 en d9 (d0 is start proefperiode) van elke proefperiode zijn insuline profielen bepaald tot 372 min (6,2u) na het voeren; daarnaast zijn op elke d9 glucose profielen bepaald. Op d2, d5 en d9 werd tevens een bloedmonster genomen voor de bepaling van IGF-1.
Om het effect van een hoge voerfrequentie (=kleine portiegrootte) te onderzoeken (zie experiment 1), is daarnaast gekeken naar het effect van een 0,5kg portie van het DL voer (wat overeenkomt met 1/6 portie van de dagelijkse hoeveelheid, omdat in experiment 1 het DL voer 6x/dag gevoerd

werd met intervallen van 4u) op insuline afgifte. Op d3 werd daarvoor een 0,5kg portie van het DL voer gevoerd, en zijn insuline profielen bepaald tot 4u na het voeren.

Voorlopige resultaten

Glucose profielen en parameters (basaal glucose, AUC/6,2u en gemiddelde glucose niveau) waren vergelijkbaar voor alle proefvoeders. Insuline profielen voor de verschillende proefvoeders zijn weergegeven in figuur 1 en insuline parameters in Tabel 1. Insuline profielen en parameters verschilden niet tussen de verschillende dagen (d2, d5 en d9).

De proefvoeders D, DL en SL resulteerden in de hoogste insuline respons (hoogste pieken, gemiddeldes en AUC/6,2u). De insuline profielen en parameters voor de voeders S, L en DSBP weken niet significant af van het controle voer.



Figuur 1. *Insuline profielen (LSmeans; gemiddelde van d2, d5 en d9) rondom de 8u voerbeurt voor de verschillende proefvoeders ten opzichte van het controle voer (n=aantal zeugdagen).*

Tabel 1. *Insuline parameters (gemiddelde van d2, d5 en d9) voor de verschillende proefvoeders (LSmeans±SE).*

	CON	**D**	**DL**	**S**	**L**	**SL**	**DSBP**	**P-waarde[1]**
Aantal zeugen	*9*	*7*	*9*	*8*	*10*	*8*	*7*	
Aantal zeugdagen	*27*	*20*	*25*	*20*	*28*	*23*	*20*	
Basaal, µU/ml	8,7±0,5	8,7±0,5	9,4±0,5	9,8±0,5	7,9±0,5	9,6±0,5	9,0±0,5	0,04
Piekhoogte, µU/ml	38±5^{a}	53±5^{ab}	57±5^{b}	43±5^{ab}	43±5^{ab}	56±5^{b}	46±5^{ab}	<0,01
Stijging, µU/ml	29±5^{a}	45±5^{ab}	47±5^{b}	33±5^{ab}	34±5^{ab}	46±5^{b}	37±5^{ab}	<0,01
Gemiddelde, µU/ml	16±1^{a}	20±1^{b}	20±1^{b}	18±1^{ab}	16±1^{a}	19±1^{ab}	18±1^{ab}	<0,01
AUC/6,2u, µU	2842±310	4137±357	3801±326	3001±359	2979±313	3495±337	3501±357	0,02

[1] Statistische significantie; het effect van dag en de interactie proefvoeder*dag was niet significant (P>0,05); [ab] Waarden met verschillende letter binnen dezelfde rij zijn significant verschillend (P<0,05).

Gemiddelde IGF-1 niveaus zijn weergegeven in Tabel 2. Verschillen in IGF-1 niveaus tussen de verschillende proefvoeders waren klein. Het DSBP voer resulteerde in de laagste IGF-1 niveaus. Het CON, L en SL voer resulteerde in de hoogste IGF-1 niveaus. IGF-1 niveaus voor de voeders D, DL en S lagen er tussenin.
Daarnaast was er een significant dageffect (P<0,05); IGF-1 niveaus waren hoger op d2 dan op d5 en d9 (LSmeans waren respectievelijk 156, 150 en 150 ng/ml voor d2, d5 en d9).

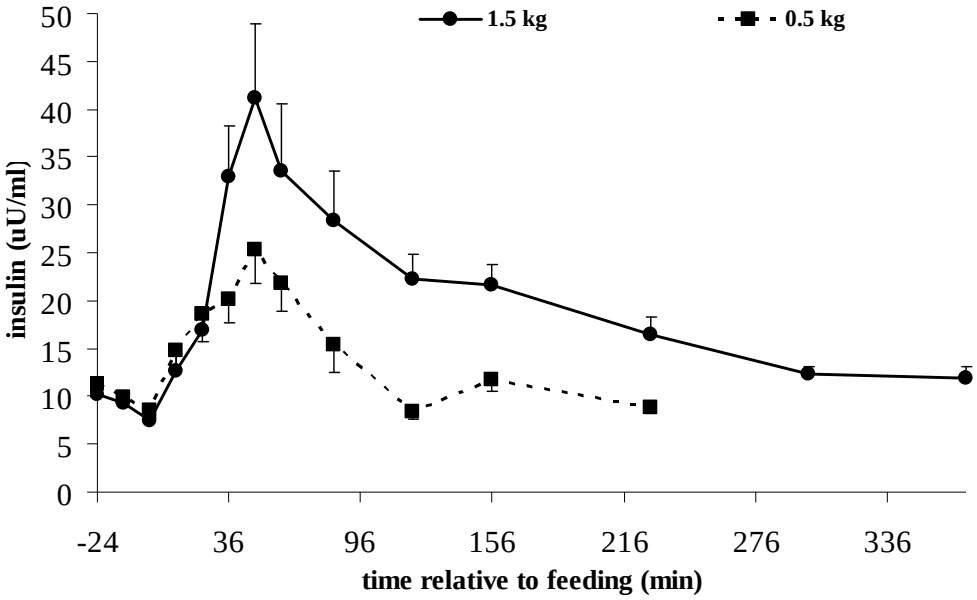
Tabel 2. *IGF-1 niveaus (gemiddelde van d2, d5 en d9) voor de verschillende proefvoeders (LSmeans±SE).*

	CON	**D**	**DL**	**S**	**L**	**SL**	**DSBP**	**P-waarde[1]**
Aantal zeugen	*9*	*8*	*10*	*8*	*10*	*9*	*8*	
Aantal zeugdagen	*27*	*23*	*26*	*19*	*30*	*18*	*21*	
IGF-1, ng/ml	156±12^{b}	153±12^{ab}	151±12^{ab}	150±12^{ab}	157±12^{b}	155±12^{b}	142±12^{a}	0,01

[1] Statistische significantie; het effect van dag was significant (P<0,05); de interactie proefvoeder*dag was niet significant (P>0,05); [ab] Waarden met verschillende letter binnen dezelfde rij zijn significant verschillend P<0,05).

Effect kleine portie DL

Het effect van een 0,5kg portie (1/6 van de dagelijkse hoeveelheid) ten opzichte van een 1,5kg portie van het DL voer is weergegeven in figuur 2. De insuline AUC na een 0,5kg portie van het DL voer was 869±187 µU/4u. Dit zou overeen komen met 2607 µU/12u, veronderstellende dat het DL voer elke 4u gevoerd werd en bij elke voerbeurt dezelfde insuline respons zou opleveren. Voor de 1,5kg portie is het insuline niveau bepaald tot 6,2u na voeren, maar om deze respons te kunnen vergelijken met de insuline respons na de kleine portie, is aangenomen dat het insuline niveau tussen 6,2u na voeren en 12u na voeren in een rechte lijn daalt naar basaal niveau (dit lijkt aannemelijk op basis van de insuline profielen uit experiment 1, zie figuur 2 in hoofdstuk 1). De AUC/12u is dan 4328±509 µU, wat beduidend hoger is dan de totale insuline-afgifte bij het 6x/dag voeren van kleine porties.



Figuur 2. *Effect van een 0,5kg portie vs. een 1,5kg portie van het DL voer op insuline afgifte*

Discussie

De voeders met dextrose (D), dextrose plus lactose (DL) en sucrose plus lactose (SL) lijken de hoogste potentie te hebben om insuline-afgifte te stimuleren. Het is echter onbekend waarom zowel lactose (L) als sucrose (S) als afzonderlijke componenten niet insuline-stimulerend lijken te werken ten opzichte van het controle voer. Daarnaast bestaat de mogelijkheid dat effecten van lactose en suikerbietenpulp op insuline-afgifte pas na 6u zichtbaar worden (insuline niveaus zijn in deze studie bepaald tot 372min (ofwel 6,2u) na voeren), omdat het enige tijd kost voordat het voer de dikke darm bereikt heeft, gefermenteerd wordt en fermentatieproducten gebruikt worden voor gluconeogenesis. Het is verder onduidelijk waarom het DSBP voer (met daarin maar liefst 80g/kg dextrose) niet resulteerde in een hoge insulinepiek direct na voeren.

Het effect van de verschillende voeders op IGF-1 niveaus lijkt klein.

Het insuline-stimulerende effect van voeders (in dit geval getest voor het DL voer) neemt af bij frequenter voeren (ofwel kleinere porties). De relatief lagere insuline respons na een kleinere voerportie hangt waarschijnlijk samen met de verlaagde afgifte van insuline-stimulerende darmhormonen zoals CCK en GLP-1, al dan niet gecombineerd met een vertraagde maaglediging.

3. ALGEMENE CONCLUSIE

Uit experiment 1:

- Insuline niveaus tijdens het interval spenen-ovulatie zijn gerelateerd aan zowel follikelontwikkeling en LH afgifte, als aan luteale ontwikkeling, progesteron afgifte en embryonale ontwikkeling tijdens de vroege dracht. Luteale ontwikkeling (corpus luteum diameter en gewicht) is daarbij ook direct gerelateerd aan follikelontwikkeling;

- Insuline niveaus tijdens het interval spenen-ovulatie beïnvloeden wel de algehele ontwikkeling van embryo's, maar lijken niet specifiek de uniformiteit van de embryo's te beïnvloeden. De consequenties hiervan voor de verdere dracht en uniformiteit bij geboorte moeten nader onderzocht worden;

- De behandeling had effect op follikelontwikkeling, de hoogte van de LH piek, luteale ontwikkeling en embryo ontwikkeling, wat er op kan duiden dat naast de absolute dagelijkse insuline-afgifte, het patroon waarin insuline afgegeven wordt een additioneel effect heeft; 2 langdurige insuline pieken per dag leiden tot betere follikelontwikkeling, een hogere LH piek, beter ontwikkelde corpora lutea en beter ontwikkelde embryo's vergeleken met frequentere, maar kortere insuline pieken.

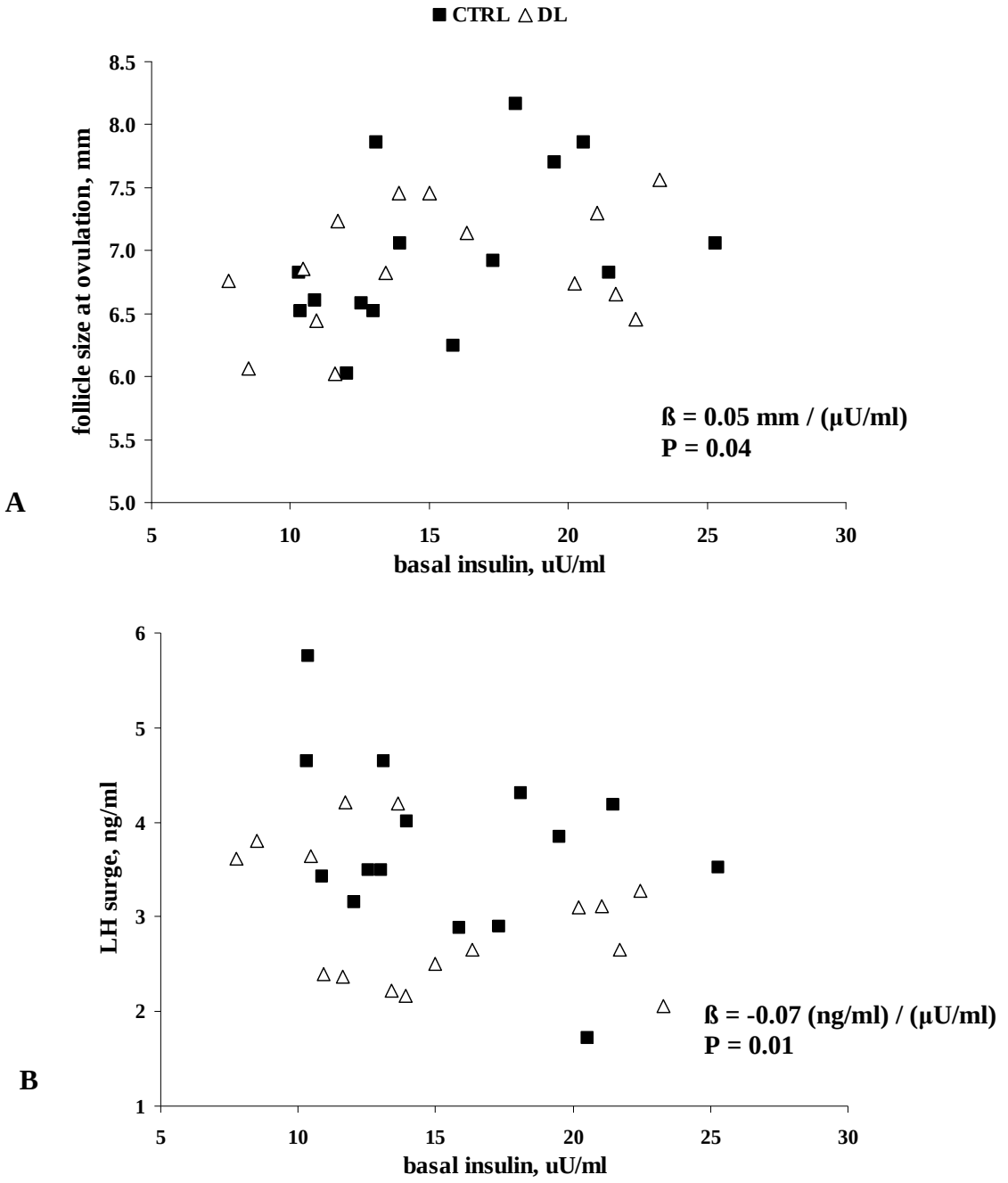
Uit experiment 2:

- Als voeders 2x/dag gevoerd worden, hebben de voercomponenten dextrose en sucrose, in combinatie met lactose, de hoogste potentie om insuline-afgifte te stimuleren (snelle, hoge pieken);

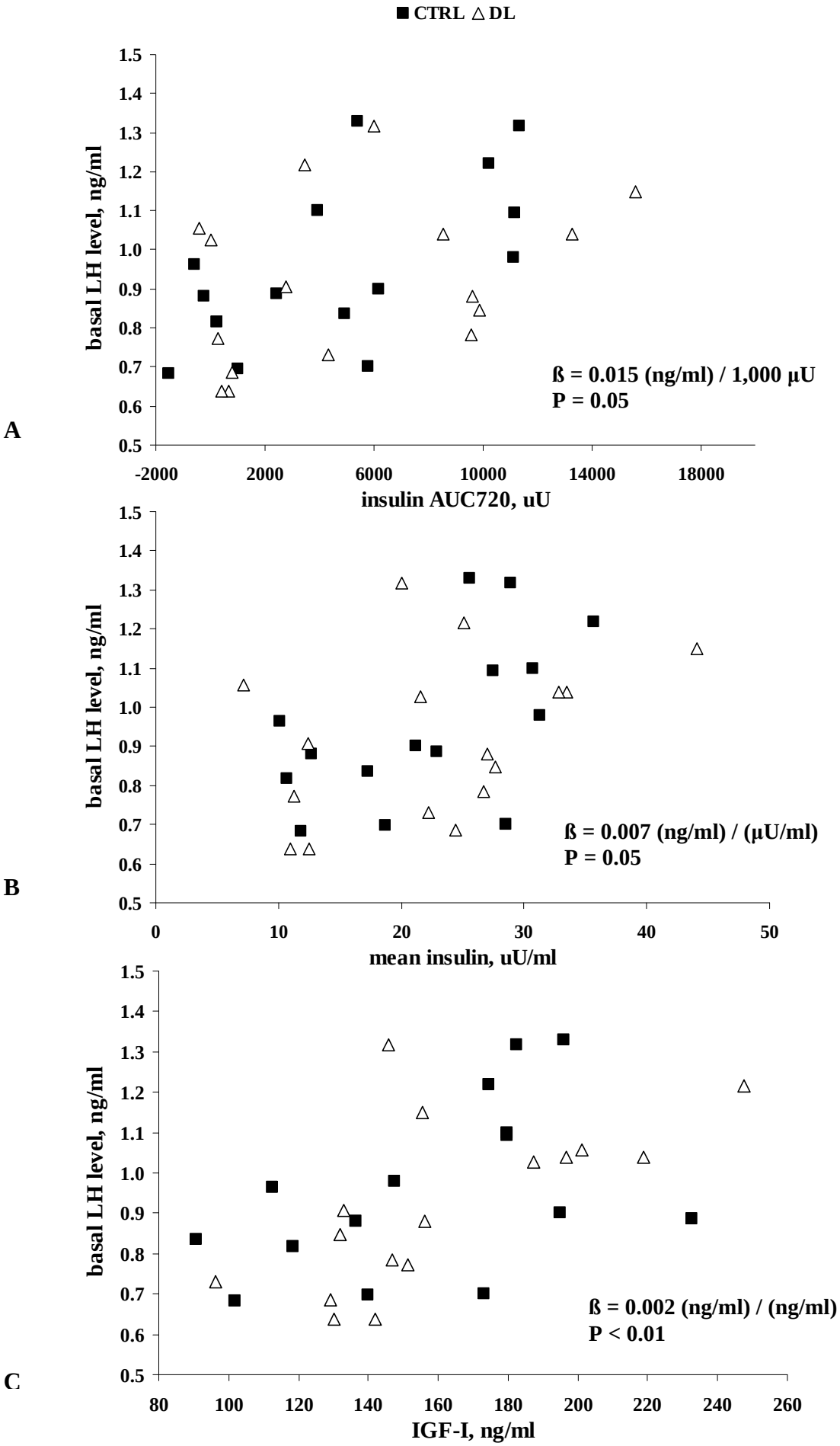
- Wanneer ook de langdurige insuline-afgifte van belang is voor de beïnvloeding van follikelontwikkeling, verdienen ook fermenteerbare NSP's (zoals lactose en suikerbietenpulp) nadere bestudering; in het huidige experiment is daarvoor wellicht het insuline verloop te kort gevolgd (tot 6,2u na voerbeurt).

BIJLAGE A. FIGUREN RELATIES INSULINE/IGF-1 MET REPRODUCTIE KENMERKEN

(*Figuren 1 en 2 zijn afkomstig uit paper deel I)*

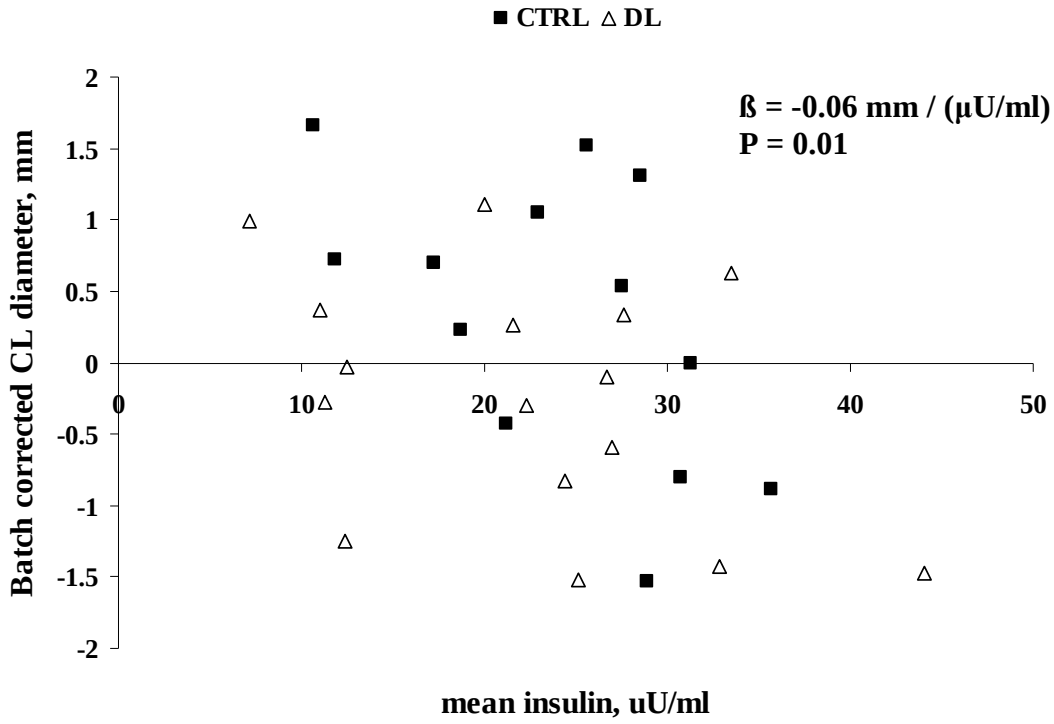


Figuur 1. *Relaties tussen basaal insuline (gemiddelde van d2 en d3na spenen) met A). follikel diameter bij ovulatie; en B). LH piekhoogte (absolute LH piekhoogte was significant hoger in CTRL vergeleken met DL: 3,73 vs. 3,00 ng/ml; P=0,03).*

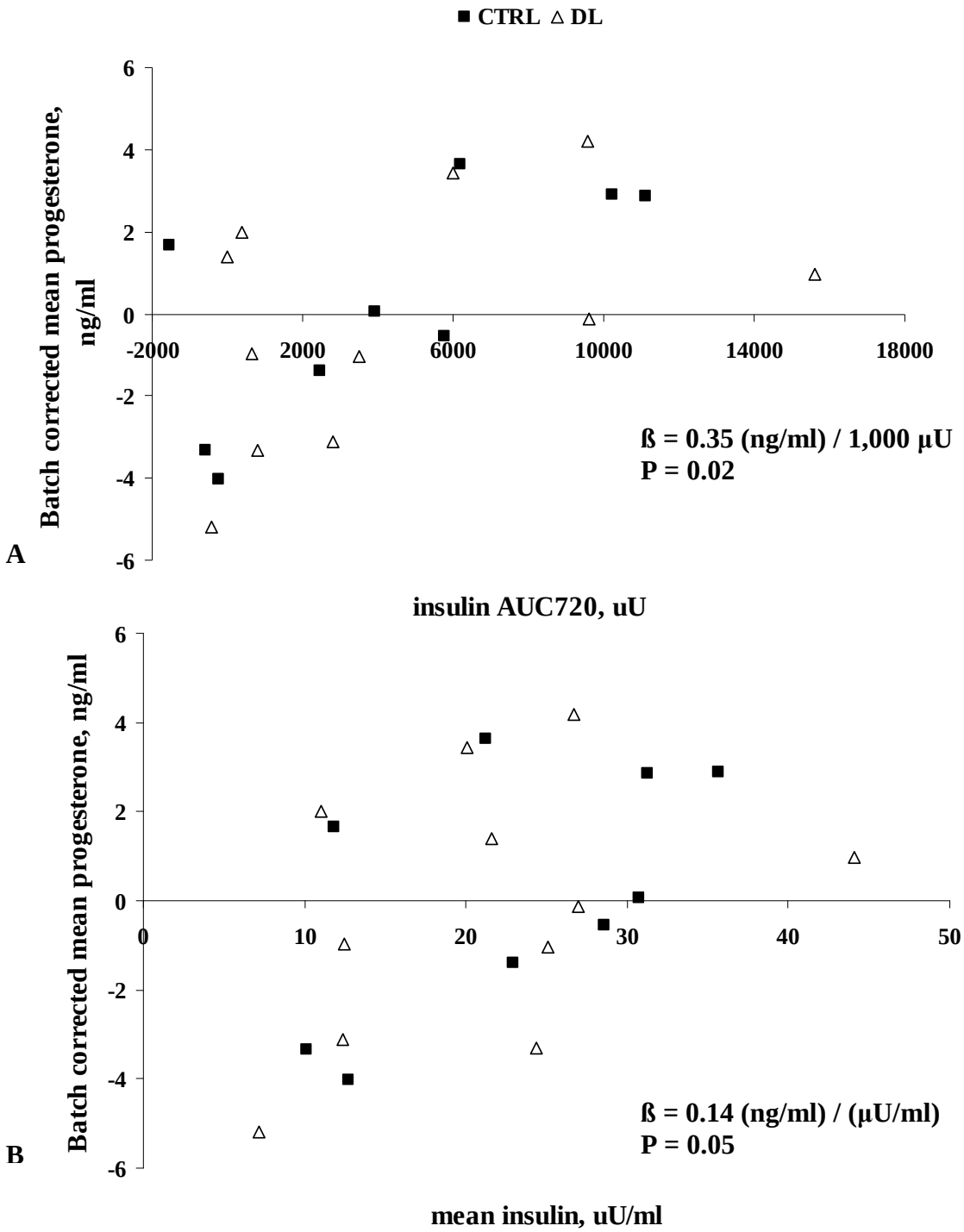


Figuur 2. *Relaties tussen A). insuline AUC/12u (gemiddelde van d2 en d3 na spenen); B). gemiddeld insuline niveau (gemiddelde van d2 en d3 na spenen); en C). IGF-1 (gemiddelde niveau gedurende d3-5 na spenen) met basaal LH niveau.*

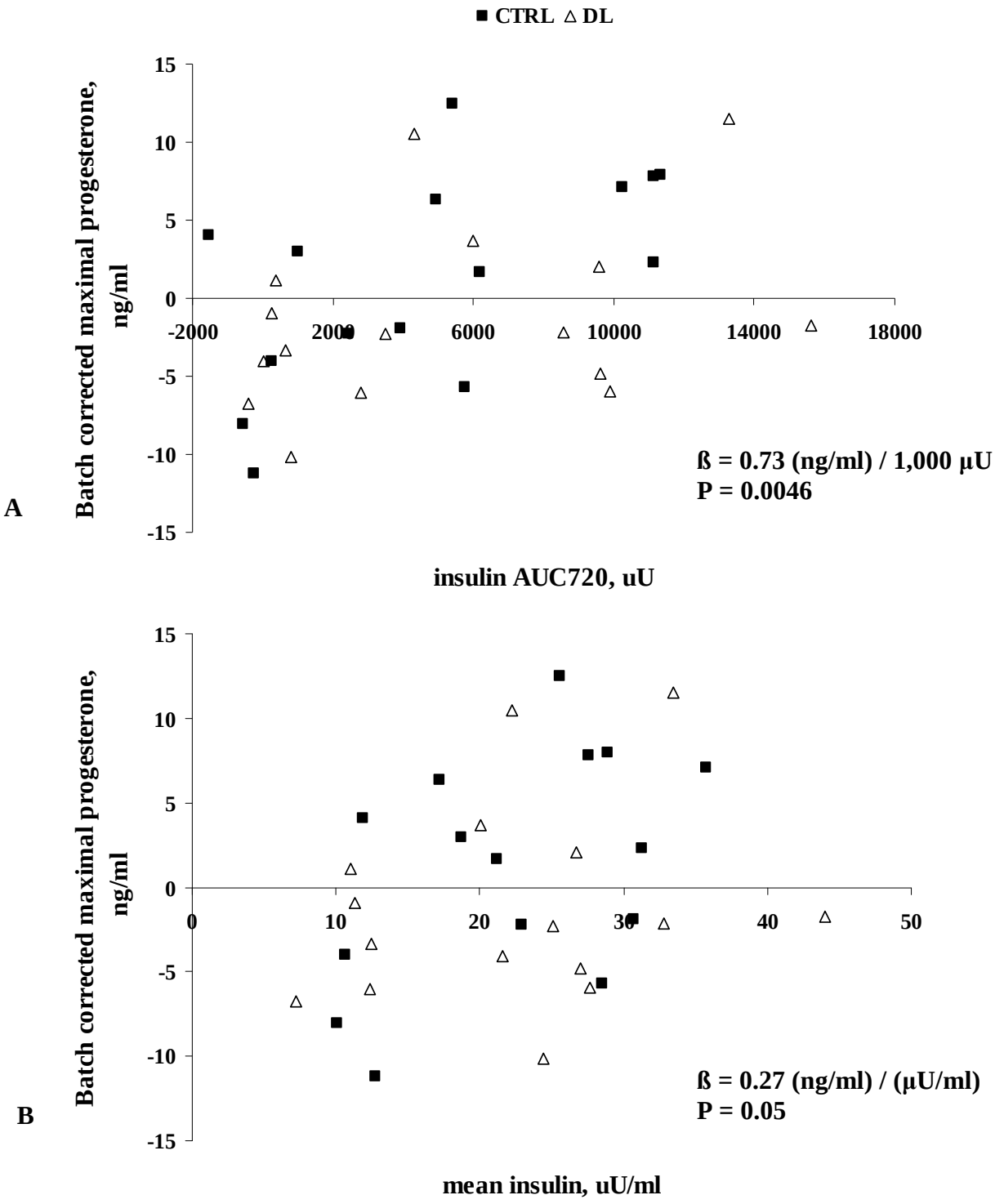
Figuren 3 t/m 6 zijn afkomstig uit paper deel II



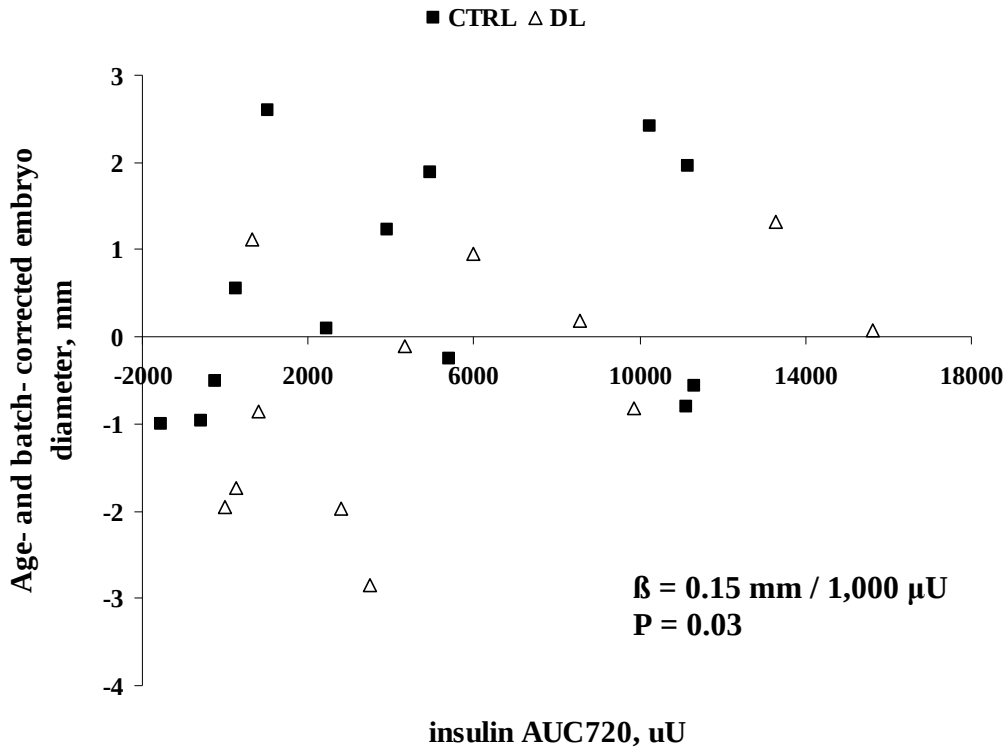
Figuur 3. *Relatie tussen gemiddeld insuline niveau (gemiddelde van d2 en d3 na spenen) met CL diameter (residuals gecorrigeerd voor een batch-effect; LSmeans waren respectievelijk 10,5, 9,6 en 9,6 mm voor batch 1, 2 en 3; P=0,04)*



Figuur 4*. Relatie tussen A). insuline AUC/12u (gemiddelde van d2 en d3 na spenen) en B). gemiddeld insuline niveau (gemiddelde van d2 en d3 na spenen) met gemiddeld progesteron niveau gedurende de eerste 10d van de dracht (residuals gecorrigeerd voor een batch-effect; LSmeans waren respectievelijk 18,14, 13,92 en 12,80 ng/ml voor batch 1, 2 en 3 P<0,01)*



Figuur 5. *Relatie tussen A). insuline AUC/12u (gemiddelde van d2 en d3 na spenen) en B). gemiddeld insuline niveau (gemiddelde van d2 en d3 na spenen) met maximaal progesteron niveau op d10 van de dracht (residuals gecorrigeerd een batch-effect; LSmeans waren respectievelijk 37,47, 28,84 en 26,04 ng/ml voor batch 1, 2 en 3; P<0,01)*



Figuur 6. *Relatie tussen insuline AUC/12u (gemiddelde van d2 en d3 na spenen) en embryo diameter (residuals gecorrigeerd voor het effect van batch en embryo-leeftijd; LSmeans waren respectievelijk 7,9, 5,9 and 6,0 voor batch 1, 2 en 3 (P=0,04); LSmeans waren 5,4 en 7,7 voor 9,5 en 10d-oude embryo's, respectievelijk (P=0,0012)*

BIJLAGE B. PAPER EXPERIMENT 1 - DEEL 1

Running head: Insulin and follicle development in sows

Title: Diet composition and feeding frequency during the weaning-to-ovulation interval in multiparous sows: I. Effects on insulin, IGF-1, luteinizing hormone and on follicle development, estrus and ovulation[1]

Authors: J.G.M. Wientjes[2], N.M. Soede, B.F.A. Laurenssen, R.E. Koopmanschap, H. van den Brand and B. Kemp

Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700AH, Wageningen, The Netherlands

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[2] To whom correspondence should be addressed: P.O. Box 338, 6700AH (phone: +31 317 486328; fax: +31 317 485006; E-mail: anne.wientjes@wur.nl).

ABSTRACT: To get more insight in how insulin secretion patterns, and corresponding IGF-1 levels, are related to LH secretion, (uniformity in) follicle development and ovulation, 32 multiparous sows were fed either a dextrose plus lactose-containing diet (DL; each 150 g/d) at 4h intervals or an isocaloric and isonitrogenous control diet at 12h intervals (CTRL; containing soybean oil) during the weaning-to-ovulation interval (WOI). At d2 and 3 after weaning insulin profiles were determined around the 0800h feeding. IGF-1 levels were determined in 0800h plasma samples at d1, 2, 3, 4 and 5 after weaning. At d4 after weaning, number and diameter of all follicles ≥ 3 mm were measured using ultrasound. Insulin parameters (basal, AUC over 12h (AUC720) and mean insulin) were similar for both treatments, but the secretion pattern differed (6 short peaks vs. 2 long peaks per day). IGF-1 levels during the WOI were similar for both treatments. Weaning-to-estrus interval, estrus duration, WOI and ovulation rate were not influenced by treatment. LH surge level was higher in CTRL sows compared with DL sows (3.73 vs. 3.00 ng/ml; $P = 0.03$). Number of follicles ≥ 3 mm at d4 after weaning was comparable between treatments, but average diameter of these follicles tended to be higher in CTRL sows (6.5 vs. 6.1 mm; $P = 0.08$) and uniformity of these follicles was higher in CTRL sows compared with DL sows (SD: 0.72 vs. 0.88 mm, $P = 0.05$; CV: 11 vs. 15%, $P = 0.02$). Basal insulin (mean value of d2 and 3 after weaning) was positively related with follicle diameter at ovulation ($P = 0.04$) and negatively related with LH surge level ($P = 0.01$). Insulin AUC720 (mean value of d2 and 3 after weaning; $P = 0.05$), mean insulin (mean value of d2 and 3 after weaning; $P = 0.05$) and mean IGF-1 level during d3-5 after weaning ($P < 0.01$) were positively related to basal LH level around the LH surge. Insulin and IGF-1 parameters were not related to the number, diameter and uniformity of follicles ≥ 3 mm at d4 after weaning. From these data, it can be concluded that insulin and IGF-1 levels during the WOI are related to LH secretion and follicle development. Feeding strategies (feeding frequency and diet composition) which modulate the pattern of insulin secretion, without affecting daily insulin output or IGF-1, can influence LH secretion and development and uniformity of pre-ovulatory follicles.

Key words: Follicle development, insulin-like growth factor-1 (IGF-1), insulin, luteinizing hormone (LH), nutrition, sows

INTRODUCTION

Recent studies have shown that specific feed components as dextrose and lactose (Van den Brand et al., 2006; 2009) or sugarbeet pulp (Ferguson et al., 2006) in pre-mating sow diets can affect uniformity of fetuses and piglets. Those effects may be mediated through the insulin-stimulating effect of these diets. Insulin and IGF-1 are known to stimulate follicle and oocyte development, either indirectly at the brain level via stimulation of LH (Koketsu et al., 1996;

Van den Brand et al., 2001), or directly at the ovarian level (Poretsky and Kalin, 1987; Quesnel et al., 2007).

It is unknown which insulin profiles are optimal for good follicle quality and uniformity, and how these insulin profiles can be achieved. Positive relationships between insulin and LH were found in studies in which sows were fed hourly (Tokach et al., 1992; Koketsu et al., 1996). In studies with twice a day feeding, however, these relationships were less clear (e.g. Van den Brand et al. (2000)). This indicates that besides the absolute amount of insulin secreted, also the pattern in which insulin is secreted (e.g. prolonged enhanced insulin levels) could play a role in LH stimulation, and thereby follicle development. Besides feeding frequency, insulin secretion pattern can also be modulated by diet composition; e.g. starch- plus dextrose-rich diets resulted in a faster, higher and longer insulin peak after feeding compared with fat-rich diets in gilts and sows (Van den Brand et al., 1998; 2001; Zieçik et al., 2002), and sugarbeet pulp enhanced insulin levels for a prolonged period after feeding in gilts (Vestergaard, 1997).

To get more insight in how different insulin secretion patterns, and corresponding IGF-1 levels, are related to LH surge characteristics, (uniformity in) follicle development and ovulation, sows were fed either a dextrose plus lactose-containing diet at 4h intervals or an isocaloric and isonitrogenous control diet at 12h intervals during the weaning-to-ovulation interval (WOI).

MATERIALS AND METHODS

General Design

During the WOI, multiparous sows were fed either a dextrose plus lactose-containing diet (DL) at 4h intervals or an isocaloric and isonitrogenous control diet (CTRL; dextrose and lactose were exchanged by soybean oil) at 12h intervals from day of weaning until ovulation. Two factors, specific feed components (dextrose plus lactose) and a high feeding frequency, were used simultaneously to create large contrasts in insulin and IGF-1 levels and patterns among sows, and in an attempt to keep insulin levels enhanced and more constant for a prolonged period of the day. After ovulation, all sows received a standard gestation diet at 12h intervals until slaughter at d10 after ovulation. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, The Netherlands).

Animals and Housing

Multiparous (parity 5.9±0.3; range 3-9) Topigs 20 (Topigs, Vught, The Netherlands) sows (n = 38), from one registered sow farm, arrived at the experimental farm of Wageningen University within 2h from weaning (d0), in 3 consecutive batches (11, 13 and 14 sows, respectively). At the day of weaning, sows received either a permanent jugular vein catheter (23 sows) for blood sampling during the whole experimental period, or an ear vein catheter

(15 sows) for blood sampling only during the WOI. Jugular vein catheters were surgically fitted under general anaesthesia as described by Soede et al. (1997). For insertion of the ear vein catheters, sows were fixated by a nose-sling. A 1.75 m catheter (medical PVC tube, inner diameter 0.8 mm, outer diameter 1.6 mm; Rubber BV, Hilversum, The Netherlands) was inserted 50 cm into the ear vein. The other end of the catheter was passed externally to the back of the sow and a one-way luer-lock stopcock (Vygon, Veenendaal, The Netherlands) was secured. To protect the catheter from damage, the ear was fixated at the head of the sow, and the catheter was fixated at the neck and back of the sow (with belts tied around the neck and chest). After insertion of the ear vein catheter, sows were given 2.2 mg Flunixine kg^{-1} i.m. (Intervet Schering-Plough Animal Health, Boxmeer, The Netherlands). Ear vein catheters were removed after ovulation.

Sows were housed in individual farrowing crates during the whole experiment. Sows were exposed to 16h of light (0700-2300) and barn temperature was maintained between 18 and 22 ºC during the whole experiment.

Sows were weighed and P2 backfat was measured within 3d after farrowing (at the sow farm), at weaning and at slaughter at d10 of pregnancy. Total litter weight was measured at weaning.

During the experiment, 6 sows (4 CTRL, 2 DL) were veterinary treated for pneumonia (5 sows) or diarrhea (1 sow) and were excluded from all analyses. The remaining sows (n = 32) had an average body weight at weaning of 251±5 kg, a lactational body weight loss of 12.0 ± 0.5%, a backfat thickness at weaning of 15 ± 0.5 mm, and a lactational backfat loss of 24.5 ± 1.3%. Furthermore, lactation length was 25.1 ± 0.1 d, number of weaned piglets was 11.6 ± 0.1 piglets and litter weight at weaning was 86 ± 2 kg.

Dietary treatments

Treatments consisted of 2 different feeding regimes during the WOI (from weaning until 12h after ovulation), to create contrasts in insulin and IGF-1 levels and patterns among sows. Within 2 parity classes (≤ 5 or ≥ 6), sows were ranked according to lactational body weight loss (%) and alternately assigned to 2 dietary treatments: a dextrose plus lactose-containing diet (DL) fed at 4h intervals, or a control diet (CTRL) fed at 12h intervals (0800h and 2000h).

Either dextrose plus lactose (each 150 g/d), or soybean oil (108 g/d) was added to a basal diet with sufficient protein, vitamins and minerals (Table 1). Both diets (manufactured by Research Diet Services BV, Wijk bij Duurstede, The Netherlands) were fed to be isocaloric and isonitrogenous. At day of weaning, sows received 1,000 g of either the DL-diet or the CTRL-diet (at 1800h). From d1 after weaning (0800h) the DL-diet was fed in 6 equal portions of 633 g (3,800 g/d). The CTRL-diet was fed in 2 equal portions of 1,800 g (3,600 g/d). From 12h after ovulation until slaughter, all sows were fed the basal diet (3,000 g/d) at 12h intervals (0800h and 2000h). Water was available ad libitum during the whole experiment.

At 1h after feeding, feed refusals were removed and weighed. Refusal samples were stored at 4 ºC until analysis for dry matter content. After drying the refusal samples (ca. 10 g) for 24h at 103 ºC, dry matter refusal was calculated by multiplying the dry fraction of the sample with the total refusal weight, and DMI was calculated by subtracting the dry matter refusal from the dry matter offered.

Blood Sampling

From d1 after weaning (0800h) until time of ovulation, blood samples were taken at 4h intervals for all sows. IGF-1 levels were determined in 0800h plasma samples at d1, 2, 3, 4 and 5 after weaning. Fourteen plasma samples taken at 4h intervals around the expected LH surge (from 54h until 2h before time of ovulation) were analyzed for LH levels.

Furthermore, at d2 and 3 after weaning, blood samples were taken at -12, 0, 12, 24, 36, 48, 60, 84, 120, 156 and 240 min (for all sows) and at 360, 480 and 720 min (only for CTRL sows, because DL sows were fed again at 1200h) relative to 0800h feeding for determination of glucose and insulin profiles.

Blood samples were collected in polypropylene tubes containing 100 µl EDTA solution (144 mg/mL saline; Tritiplex III, Merck Nederland B.V., Amsterdam, The Netherlands), immediately placed on ice after collection and centrifuged at 1,710 x *g* for 10 min at 4 ºC. Plasma was stored at -20 ºC until analyses.

Follicle development, estrus and ovulation

On d0 follicle diameter was determined with transrectal ultrasonography (Scanner 200, Pie Medical/Esaote, Maastricht, The Netherlands), by averaging the diameter of the 5 largest follicles at one ovary.

From d2 after weaning (0800h), estrus detection was performed at 4h intervals (after feeding and blood collection) by a back-pressure test in the presence of one of 2 mature vasectomized boars. Time of onset of estrus was defined as 2h before the first time a sow showed a standing response; end of estrus was defined as 2h after the last time the sow showed a standing response.

At d4, ultrasound clips of both complete ovaries were made, using a Mylab 30 scanner (Pie Medical/Esaote, Maastricht, The Netherlands). Diameter and number of antral follicles was analyzed using frame by frame analysis; for each individual follicle the largest diameter of several (≥ 3) consecutive frames was measured. Using this method, follicles larger than 3 mm can be reliably assessed (N.M. Soede, unpublished results).

From 12h after onset of estrus sows were transrectally scanned at 12h intervals (0800h and 2000h) to determine time of ovulation (using the scanner 200). The 5 largest follicles at one ovary were measured. Time of ovulation was defined as 6h before the first scanning time that no large antral follicles were identified anymore. When substantial fewer large antral follicles were identified than before, ovulation was assumed to have just started and time of ovulation

was defined as time of scanning. Ovulation was confirmed by a further scan 12h later. Follicle diameter at ovulation was defined as the mean diameter of the 5 largest follicles at the last scanning before ovulation started.

Sows were inseminated every day of estrus with a commercial dose of semen (containing 2 x 10^9 sperm cells) of a Topigs boar line, until ovulation had occurred.

Sows were slaughtered at the experimental farm 10d after ovulation and reproductive tracts were removed. The number of corpora lutea was counted on both ovaries. Data on uteri, luteal development and embryos are published elsewhere (Wientjes et al., submitted).

Plasma Analyses

Glucose and insulin. For glucose analyses, 500 µL 0.3M Trichloroacetic Acid (TCA) was added to 50 µL of plasma for precipitation of protein. After centrifugation at 16,000 x *g* for 1 min, glucose levels in the supernatant were analyzed in triplicate with an enzymatic colorimetric assay using the glucose-oxidase-peroxidase (GOD-PAP) method using a commercial kit (Roche Diagnostics Nederland BV, Almere, The Netherlands). Plasma insulin levels were analyzed in duplicate with a commercial RIA-kit (PI-12K Porcine Insulin RIA-kit, Millipore, St. Charles, USA). The sensitivity was 2 µU/ml, and intra- and interassay CV were 6.4% (n = 42) and 6.0% (n = 9), respectively.

For each sampling day, basal glucose and basal insulin levels were calculated as the mean value of the 2 samples taken before feeding (-12 and 0 min); maximal insulin levels were defined as the maximum value during the first 156 min after feeding (when no values from t = 12 until t = 156 min were above basal level, no maximum levels were defined (n = 3 sow days)); the increase in insulin after feeding was calculated as the difference between maximal and basal levels; the area under the curve (AUC) during the sampling period was calculated as the area above basal glucose and insulin levels, where AUC240 was calculated as the area under the curve from feeding until 240 min after feeding for all sows, and AUC720 was calculated as the area under the curve from feeding until 720 min after feeding for CTRL sows, and as AUC240 multiplied by 3 for DL sows as DL sows were fed again at 1200h and 1600h; and the mean glucose and insulin level during the sampling period were calculated as the average glucose and insulin levels of all plasma samples after feeding (from 0 until 720 min in CTRL; from 0 until 240 min in DL) corrected for the time intervals between samples.

IGF-1. IGF-1 levels were quantified in duplicate, using a commercial kit (IRMA IGF-1 A15729, Immunotech, Marseille, France), after extraction of the samples with ethanol/HCl (as validated by Louveau and Bonneau (1996)). The sensitivity, intra- and interassay CV were 2 ng/ml, 2.2% (n = 26) and 3.5% (n = 12), respectively.

LH. Plasma LH concentrations were analyzed in triplicate, using the homologous double-antibody RIA, as described previously by Cosgrove et al. (1991), with the following modifications: 1% BSA was used in the assay buffer; for the precipitation 50 µL cold Saccel (anti sheep/goat, IDS-AA-SAC2; Lucron Bioproducts BV, Gennep, The Netherlands) was

used; after mixing and incubation for 1h, tubes were centrifuged at 6,240 x g for 6 min at 4ºC, aspirated and counted.
The lower limit of detection was 0.012 ng/ml; the intra- and interassay CV were 6.3% (n = 36) and 4.5% (n = 8), respectively.
Basal LH levels were calculated as the average value of the 3 lowest values of all samples (either before or after the LH surge); LH surge level was defined as the maximum value of all samples.

Statistical Analyses

Data are presented as means±SE, unless otherwise stated. Data were analyzed with the GLM procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC), unless otherwise stated. For all GLM-analyses, first interaction terms were tested and removed from the model when not significant ($P > 0.10$), followed by a stepwise removal of the variable with the highest non-significant P-value ($P > 0.10$), except for the factor treatment.

Dry matter intake. DMI was analyzed using model 1: $Y_{ijk} = \mu + T_i + B_j + T_i*B_j + e_{ijk}$, where Y_{ijk} = dependent variable; μ = overall mean; T_i = treatment (i = CTRL, DL); B_j = batch (j = 1,2,3); T_i*B_j = interaction between treatment and batch; and e_{ijk} = residual error. Pearson correlation coefficients (using the CORR procedure) were calculated between total DMI during the WOI and DMI at sampling days for glucose and insulin profiles.

Glucose and insulin. Because of our interest in the direct effect of both diets on glucose and insulin profiles, glucose and insulin profiles were only analyzed for sows with a DMI of 75% or more at both sampling days (0800h feeding at d2 and 3; n = 17), using model 2: $Y_{ijklmn} = \mu + T_i + B_j + e1_{ijk} + ST_l + SD_m + T_i*ST_l + T_i*SD_m + ST_l*SD_m + e2_{ijklmn}$, where Y_{ijklmn} = dependent variable; μ = overall mean; T_i = treatment (i = CTRL, DL); B_j = batch (j = 1,2,3); $e1_{ijk}$ = error term 1, which represents the random effect of sow_k (k = 1 to 32) nested within treatment and batch; ST_l = sampling time (l = -12, 0, 12, 24, 36, 48, 60, 84, 120, 156, 240 min); SD_m = sampling day (m = d2, d3); T_i*ST_l = interaction between treatment and sampling time; T_i*SD_m = interaction between treatment and sampling day; ST_l*SD_m = interaction between sampling time and sampling day; and $e2_{ijklmn}$ = residual error. The effect of treatment and batch was tested against error term 1. Effects of sampling time, sampling day and all interactions were tested against the residual error. Because for insulin profiles the variance of error terms was not uniform over time (variance decreases with time after feeding), statistical differences between treatments were tested for each sampling time separately.
Like glucose and insulin profiles, glucose (basal, AUC240, AUC720 and mean) and insulin parameters (basal, maximal, increase after feeding, AUC240, AUC720 and mean) were only analyzed for sows with a DMI of 75% or more at both sampling days (n = 17), using model 2, except that sampling time and its interactions were excluded from the model.

IGF-1. For analyses of IGF-1 levels during WOI, sows were divided into 2 classes based on DMI during the WOI (DMI_{WOI}; from weaning until 12h after ovulation (dietary treatment

period); where 0 = DMI_{WOI} < 75%, n = 15 sows and 1 = DMI_{WOI} ≥ 75%, n = 17 sows). IGF-1 levels were analysed using model 3: $Y_{ijklmn} = \mu + T_i + B_j + DMI_{WOI\_k} + e1_{ijkl} + SD_m + T_i*SD_m + T_i*DMI_{WOI\_k} + SD_m*DMI_{WOI\_k} + T_i*SD_m*DMI_{WOI\_k} + e2_{ijklmn}$, where Y_{ijklmn} = dependent variable; μ = overall mean; T_i = treatment (i = CTRL, DL); B_j = batch (j = 1,2,3); DMI_{WOI_k} = class of DMI from weaning until 12h after ovulation (dietary treatment period), as % of total dry matter offered (k = 0 (< 75%), 1 (≥ 75%)); $e1_{ijkl}$ = error term 1, which represents the random effect of sow_l (l = 1 to 32) nested within treatment, batch and DMI_{WOI}; SD_m = sampling day (m = d1, d2, d3, d4, d5); T_i*SD_m = interaction between treatment and sampling day; T_i* DMI_{WOI_k} = interaction between treatment and DMI_{WOI}; SD_m* DMI_{WOI_k} = interaction between sampling day and DMI_{WOI}; $T_i*SD_m*DMI_{WOI\_k}$ = interaction between treatment, sampling day and DMI_{WOI}; and $e2_{ijklm}$ = residual error. The effect of treatment, batch and DMI_{WOI} was tested against error term 1. Effects of sampling day and all interactions were tested against the residual error.

Follicle development, estrus and ovulation. One sow (DL) developed cystic ovaries (weaning-to-estrus interval: 120h; ovulation rate: 3; no embryos recovered) and was excluded from all analyses on follicle development, estrus and ovulation. One sow (DL) had a silent estrus and was excluded from analyses on weaning-to-estrus interval (WEI), estrus duration and follicle diameter at ovulation. Follicle diameter (average diameter of 5 largest follicles at one ovary at weaning, d4 and at ovulation), WEI, estrus duration, WOI, LH traits (basal, LH surge, interval weaning-to-LH surge) and ovulation rate were analyzed using model 4: $Y_{ijkl} = \mu + T_i + B_j + DMI_{WOI\_k} + T_i*DMI_{WOI\_k} + e_{ijkl}$, where Y_{ijkl} = dependent variable; μ = overall mean; T_i = treatment (i = CTRL, DL); B_j = batch (j = 1,2,3); DMI_{WOI_k} = class of DMI from weaning until 12h after ovulation, as % of total dry matter offered (k = 0 (< 75%), 1 (≥ 75%)); $T_i*DMI_{WOI\_k}$ = interaction between treatment and DMI_{WOI}; and e_{ijkl} = random error.

Follicle development and uniformity of the follicle pool (≥ 3 mm) at d4 after weaning was further analyzed on sow level, by merging output of both ovaries to a single observation. For 3 sows (1 CTRL; 2 DL) no ultrasound clips at d4 were available and therefore these sows were excluded from all analyses on follicle development at d4 after weaning; additionally, for 8 sows (5 CTRL; 3 DL) only ultrasound clips of one ovary were available, and therefore these sows were excluded from further analyses of the follicle pool at d4. Total number of follicles, average follicle diameter, SD of follicle diameter and CV of follicle diameter of the follicle pool at d4 after weaning were therefore analyzed for 20 sows (9 CTRL and 11 DL), using model 4. Additional generalized linear regression analyses (using the Glimmix procedure) were done on follicle diameter as a binary variable (0 = < 5.0 mm and 1 = ≥ 5.0 mm; 0 = < 6.0 mm and 1 = ≥ 6.0 mm; 0 = < 7.0 mm and 1 = ≥ 7.0 mm), with treatment, batch, DMI_{WOI} and the interaction between treatment and DMI_{WOI} as fixed effects and sow added as random effect (model 5), using an exchangeable correlation structure. To check whether differences in follicle development at d4 after weaning could be explained by differences in follicle diameter

at day of weaning, follicle diameter (average diameter of 5 largest follicles at one ovary) at weaning was added as a covariable to models 4 and 5.

Relations with insulin and IGF-1. Relations between insulin and IGF-1 with all reproductive parameters were analyzed by adding insulin parameters (basal insulin, insulin AUC720 or mean insulin; mean values of both sampling days per sow) or IGF-1 parameter (mean IGF-1 level during d3 to 5 after weaning per sow) as a covariate and its interaction with treatment to model 4. Additionally, Pearson correlation coefficients (using the CORR procedure) were calculated amongst the different insulin and IGF-1 parameters that were significantly related to reproductive parameters, and amongst the different reproductive parameters that were significantly related to insulin and IGF-1 parameters.

RESULTS

Dry matter intake

A large variation in DMI_{WOI} existed among sows, but DMI_{WOI} did not differ between treatments (63 ± 10% and 73 ± 6% in CTRL and DL sows, respectively; $P = 0.37$). DMI of the 0800h feedings at d2 and 3 (sampling days for glucose and insulin profiles) was 57 ± 13% and 63 ± 11% for CTRL and DL sows, respectively, at d2 ($P = 0.72$) and 68 ± 11% and 75 ± 10% for CTRL and DL sows, respectively, at d3 ($P = 0.63$). In both treatments, DMI at d2 0800h and DMI at d3 0800h were strongly correlated to DMI_{WOI} (overall correlations were r = 0.88, $P < 0.0001$ and r = 0.82, $P < 0.0001$ for d2 and 3, respectively).

Glucose, insulin and IGF-1 profiles during WOI

Glucose levels of sows with a low DMI (< 75%; on average 21 ± 10% in CTRL and 36 ± 8% in DL; n = 15) at both sampling days (0800h feeding at d2 and 3 after weaning) remained constant after feeding, and no clear postprandial insulin peak could be observed in these sows (data not shown). Glucose and insulin levels are therefore presented for sows with a DMI of 75% or more at the 0800h feedings of d2 and 3 (on average 98 ± 1% in CTRL and 98 ± 2% in DL; n = 17). Basal glucose and basal insulin levels were comparable between sows with low and high DMI at both sampling days.

Glucose. Mean glucose levels were lower in CTRL sows at d2 (80.1 ± 2.0 mg/dl; $P < 0.05$) than in CTRL sows at d3 (89.3 ± 1.2 mg/dl), DL sows at d2 (87.6 ± 2.0 mg/dl) and DL sows at d3 (86.8 ± 2.1 mg/dl; Table 2). The treatment*sampling time*sampling day-interaction was not significant ($P = 0.40$) and therefore Figure 1 shows average glucose profiles of d2 and 3 per treatment. Glucose levels were significantly lower in CTRL sows than in DL sows at 48 min (71.6 ± 2.3 and 87.2 ± 2.6 for CTRL and DL, respectively; $P < 0.0001$) and 84 min (77.2 ± 2.1 and 87.7 ± 2.8 for CTRL and DL, respectively; $P = 0.04$) postprandial.

Basal glucose, AUC240 and AUC720 did not differ between sampling days (Table 2). Basal glucose was comparable between treatments, but glucose AUC240 was lower in CTRL sows (- 1,699 mg; $P < 0.001$) and glucose AUC720 tended to be lower in CTRL sows (- 1,896 mg; $P = 0.07$; Table 2) compared with DL sows.

Insulin. Insulin profiles and parameters are presented in Figure 1 and Table 2. Insulin profiles and parameters did not differ between sampling days. Insulin levels were higher in CTRL sows at 12 min (28.3 ± 2.9 and 17.8 ± 1.6 μU/ml; $P < 0.01$), 24 min (48.4 ± 9.7 and 24.7 ± 3.3 μU/ml; $P = 0.03$) and 240 min (32.7 ± 5.7 and 15.7 ± 1.7 μU/ml; $P < 0.01$) postprandial compared with DL sows, and tended to differ at 36 min postprandial (48.0 ± 9.9 and 29.4 ± 3.7 μU/ml in CTRL and DL sows, respectively; $P = 0.09$).

Insulin AUC240 was higher in CTRL sows than in DL sows (+ 2,165 μU; $P = 0.03$), but AUC720 and other insulin parameters did not differ between treatments (Table 2).

IGF-1. IGF-1 profiles during WOI are shown in Figure 2 for sows with a low DMI_{WOI} (< 75%; on average 27 ± 8% in CTRL vs. 50 ± 7% in DL, $P = 0.05$) and high DMI_{WOI} (≥ 75%; on average 94 ± 3% in CTRL vs. 93 ± 3% in DL, $P = 0.91$). IGF-1 levels did not differ between treatments, but increased from d1 to 3 after weaning. Until d3 after weaning, IGF-1 levels were independent of DMI_{WOI} (LSmeans were 119.5, 132.9 and 154.5 ng/ml at d1, 2 and 3, respectively), but IGF-1 levels were higher at d4 and 5 after weaning in sows with a high DMI_{WOI} compared with sows with a low DMI_{WOI} (LSmeans were 152.5 and 174.9 ng/ml at d4 ($P = 0.01$) and 143.6 and 169.9 ng/ml at d5 ($P < 0.01$) for sows with low and high DMI_{WOI}, respectively).

Follicle development, estrus and ovulation

Follicle diameter (5 largest at one ovary) at weaning, at d4 after weaning and at ovulation, WEI, estrus duration, WOI and ovulation rate were not influenced by treatment, nor by DMI_{WOI} (Table 3).

Basal LH level and interval weaning-to-LH surge were also comparable for both treatments, and unaffected by DMI_{WOI} (Table 3). LH surge level was higher in CTRL sows compared with DL sows (+ 0.73 ng/ml; $P = 0.03$; Table 3). Furthermore, sows with a high DMI_{WOI} (≥ 75%) tended to have lower LH surge levels compared with sows with a low DMI_{WOI} (< 75%) (LSmeans were 3.6 vs. 3.1 ng/ml for sows with low and high DMI_{WOI}, respectively).

Number of follicles in the follicle pool (≥ 3 mm) at d4 after weaning was comparable between treatments (21.1±0.8 and 22.1±0.7 in CTRL and DL, respectively; $P = 0.39$), but average diameter of these follicles tended to be higher in CTRL sows compared with DL sows (6.5±0.2 and 6.1±0.1 mm, respectively; $P = 0.08$), and uniformity of these follicles was higher in CTRL sows compared with DL sows (SD was 0.72±0.04 and 0.88±0.06 mm ($P = 0.05$) and CV was 11±1 and 15±1% ($P = 0.02$) in CTRL and DL sows, respectively). Average diameter

of the follicles at d4 after weaning was not influenced by average follicle diameter at day of weaning and none of the follicle pool characteristics was influenced by DMI_{WOI}.

Distribution of follicles (≥ 3 mm) over 6 different diameter categories is shown in Figure 3. Further analysis of follicle diameter as a binary variable indicated that CTRL sows had significantly fewer follicles below 5.0 mm at d4 after weaning compared with DL sows (4 ± 2 vs. 13 ± 4%; $P = 0.05$). Also the number of follicles smaller than 6.0 mm was lower in CTRL sows compared with DL sows (25 ± 7 vs. 45 ± 6%; $P = 0.04$), but number of follicles larger than 7.0 mm was comparable for both treatments (27 ± 8 vs. 17 ± 5% for CTRL and DL sows, respectively; $P = 0.37$).

Relationships between insulin and IGF-1 with follicle development, estrus and ovulation

In the analyses, no interactions existed between treatment and insulin parameters ($P > 0.10$; mean values of d2 and 3) or IGF-1 level ($P > 0.10$; mean value of d3-5 after weaning), indicating that relationships were similar for both treatments, and therefore overall treatment corrected regressions are presented.

Figure 4 shows the positive relation between basal insulin and follicle diameter at ovulation (intercept: 6.18; ß: 0.05 mm / (µU/ml); $P = 0.04$; Figure 4A), and the negative relation between basal insulin and LH surge level (intercept: 4.66; ß: -0.07 (ng/ml) / (µU/ml); $P = 0.01$; Figure 4B).

Insulin AUC720 (intercept: 0.94; ß: 0.015 (ng/ml) / 1,000 µU; $P = 0.05$; Figure 5A), mean insulin (intercept: 0.85; ß: 0.007 (ng/ml) / (µU/ml); $P = 0.05$; Figure 5B) and mean IGF-1 level at d3-5 after weaning (intercept: 0.63; ß: 0.002 (ng/ml) / (ng/ml); $P < 0.01$; Figure 5C) were positively related to basal LH level.

Insulin and IGF-1 parameters were not related to estrus and ovulation parameters, or the number, diameter and uniformity (SD; CV) of the follicle pool (≥ 3 mm) at d4 after weaning.

Correlations amongst the different insulin and IGF-1 parameters that were significantly related to one or more reproductive parameters were calculated. Basal insulin and insulin AUC720 were not correlated ($P = 0.22$), but mean insulin was highly correlated with both basal insulin (r = 0.69; $P < 0.0001$) and insulin AUC720 (r = 0.86; $P < 0.0001$). Mean IGF-1 level at d3-5 after weaning was correlated with basal insulin (r = 0.39; $P = 0.03$) and mean insulin (r = 0.39; $P = 0.03$), but not with insulin AUC720 ($P = 0.17$).

Furthermore, correlations amongst the different reproductive parameters that were significantly related to one or more insulin or IGF-1 parameters, were calculated. Follicle diameter at ovulation was not correlated with basal LH ($P = 0.34$) or LH surge level ($P = 0.98$). Basal LH level and LH surge level were positively correlated (r = 0.38; $P = 0.04$). The relative LH surge (LH surge level minus basal LH level) was not correlated with basal LH level ($P = 0.41$).

DISCUSSION

Results of this study show that in multiparous sows, insulin and IGF-1 levels during the WOI are related to LH secretion and follicle development. Basal insulin level at d2 and 3 after weaning was positively related with follicle diameter at ovulation, and negatively related with LH surge level. Both insulin AUC720 and mean insulin at d2 en 3 after weaning, and mean IGF-1 level during d3-5 after weaning were positively related to basal LH levels around the LH surge.

Relationships found between insulin and IGF-1 with basal LH levels indicate that high plasma insulin and IGF-1 levels during the WOI can stimulate the hypothalamus-pituitary-ovarian axis at brain level. The stimulating effect of insulin on pituitary LH release has been demonstrated in vitro (Adashi et al., 1981), and positive relationships between insulin and IGF-1 levels with LH secretion in lactating sows have been reported before (Tokach et al., 1992; Koketsu et al., 1996; Van den Brand et al., 2001). Beneficial effects of insulin stimulation (either by flushing or exogenous insulin injections) during the follicular phase on LH pulse frequency and ovulation rate in gilts are widely described and consistent (Cox et al., 1987; Flowers et al., 1989). Literature about effects of insulin levels (e.g. modulated by diet composition or feeding level) during only the WOI in relation to subsequent reproduction in sows, however, is scarce and inconclusive. Most studies modulated insulin levels during lactation, either or not in combination with WEI, and only measured insulin levels during lactation (Tokach et al., 1992; Koketsu et al., 1996; Van den Brand et al., 2000). Paterson and Pearce (1994) measured mean insulin levels after weaning (at d1 and 3 postweaning) in primiparous sows, but found no relationships between mean insulin levels and LH secretion or WEI. Exogenous insulin injection after weaning in primiparous sows resulted in increased follicular steroidogenesis (Whitley et al., 1998a), shorter WEI (Whitley et al., 1998b), and higher farrowing rates and litter sizes (Ramirez et al., 1997). To the authors' knowledge, relationships between post-weaning (dietary modulated) insulin and IGF-1 levels and post-weaning LH levels in multiparous sows have not been reported before.

Relationships between basal insulin levels and a) follicle diameter at ovulation and b) LH surge level, have neither been reported before, and are hard to interpret. Basal insulin levels are usually not influenced by feeding level, diet composition or feeding frequency (Kemp et al., 1995; Van den Brand et al., 1998; 2000; Zieçik et al., 2002). Pere et al. (2000) reported decreased basal insulin levels during pregnancy in multiparous sows, whereas basal insulin levels were not affected by physiological stage (pregnancy, lactation, postweaning) in primiparous sows (Pere and Etienne, 2007) or by pregnancy in gilts (Schaefer et al., 1991). This suggests that other factors determine basal insulin levels. Anticipatory neuro-physiological reflexes, for example, may increase insulin levels already before feeding (as reviewed by e.g. Power and Schulkin (2008)). It might also be possible that the low basal insulin levels in our study indicate that these sows have not yet completely obtained steady-state conditions after surgery (at day of weaning) due to fasting before surgery, in combination

with a reduced feed intake during the first days after surgery, which is for example shown by Simoes Nunes et al. (1987). Although basal insulin levels did not differ between sows with a high (≥ 75%) and low (< 75%) DMI at d2 and 3 after weaning, additional analyses showed a significant correlation between average DMI during the first 2d after surgery with basal insulin levels at d2 and 3 ($r = 0.50$; $P < 0.01$), which supports the hypothesis that steady state has not yet been reached. Additionally, basal insulin levels at d2 and 3 were correlated with mean insulin levels and with IGF-1 levels (during d3-5 after weaning), and it therefore remains speculative whether relationships between basal insulin and reproduction characteristics are causal or not.

Furthermore, the importance of pre-ovulatory LH surge levels and basal LH levels around this LH surge for subsequent oocyte and embryo development are unknown. A suboptimal LH surge level, as can be found with short lactation lengths or intermittent suckling regimes (Gerritsen et al., 2008), may lead to inadequate luteinization of the corpora lutea, and consequently reduced plasma progesterone levels and increased embryo mortality (Einarsson and Rojkittikhun, 1993). But whether further increased LH surge levels or increased basal LH levels around the LH surge have a beneficial effect on subsequent oocyte and embryo development is not clear.

The relationships between insulin and IGF-1 with follicle development and LH secretion were established in sows with 2 different feeding regimens during the WOI. Insulin secretion can be modulated by diet composition and feeding frequency. These regimens were used to create a large contrast in insulin patterns between sows, in order to get more insight in how different insulin secretion patterns and corresponding IGF-1 levels during WOI are related to LH secretion, follicle development and ovulation. Dextrose was used to stimulate a quick and high increase in insulin directly after feeding, as shown by Van den Brand et al. (1998) and Zieçik et al. (2002). Lactose was assumed to increase insulin levels for a prolonged period after feeding; adult sows have a reduced lactase activity in the small intestine (Kim et al., 1978), and consequently lactose was assumed to be fermented in the large intestine. The increase of lactobacilli concentrations in the large intestine after feeding lactose to finishing pigs found by Pierce et al. (2006), suggests that dietary lactose indeed increases fermentation activity. Also other fermentable NSP sources (sugarbeet pulp) enhanced insulin levels for a prolonged period after feeding in studies of Vestergaard (1997). Feeding 2 times a day results in higher and longer lasting insulin peaks, but also relatively long periods of low insulin levels. In an attempt to keep insulin levels enhanced and more constant for a prolonged period of the day, we fed the DL diet at a high feeding frequency (6x/d). However, at this higher feeding frequency, resulting in smaller meal sizes (633 g for DL), the DL diet did neither result in a higher total insulin secretion (AUC720, mean insulin) nor in a more sustained period of increased insulin levels compared with the CTRL diet. Instead, AUC240 was higher in CTRL sows, probably related to the larger meal size (1,800 g for CTRL).

In this study, it is not possible to separate effects of diet composition and feeding frequency (i.e. meal size). Recently (Wientjes et al., unpublished results), we fed multiparous sows (n = 9; recovered from lactation and under daily altrenogest treatment to prevent estrus and corresponding feed refusals) comparable DL and CTRL diets in 2 equal portions per day (1.5 kg per portion) in a latin square design, and found a higher postprandial insulin AUC in the DL diet compared with the CTRL diet (3,801 ± 326 vs. 2,842 ± 310 μU / 6.2h for the DL and CTRL diet, respectively). This indicates that when both diets are fed in 2 equal portions per day, the DL diet indeed results in a higher postprandial insulin response compared with the CTRL diet. When the same sows were fed a 0.5 kg portion (reflecting 1/6 of the daily amount) of the DL diet, the postprandial insulin AUC was only 869 ± 187 μU/4h (which would amount to 2,607 μU/12h, assuming that the DL diet would be fed every 4h and would result in similar insulin responses after each meal), whereas the insulin response after a 1.5 kg portion of the DL diet was 4,328 ± 509 μU/12h. Therefore, the insulin-stimulating effect of the DL diet in the current study seems to be counteracted by the effect of the smaller meal sizes (related with the higher feeding frequency). However, by modulating both diet composition and feeding frequency between treatments in this study, we created 2 completely different insulin secretion patterns (6 short vs. 2 long peaks per day), whereas the total daily insulin output was similar for both treatments.

Similar reductions in glycemic and insulinemic responses in insulin secretion with increased meal frequency were found in human (Jenkins et al., 1990; 1992). Human consumed identical diets either as 3 meals a day (at 4h intervals; three-meal diet) or 12 snacks a day (at 1h intervals; nibbling diet) (Jenkins et al., 1992). No clear postprandial glucose peaks were observed on the nibbling diet (i.e. glucose levels were flattened), and also postprandial insulin responses were reduced on the nibbling diet compared with the three-meal diet. Although total nutrient intake was similar for both diets, mean daily insulin level was 20.1 ± 5.8% reduced on the nibbling diet compared with the three-meal diet. This indicates an improved economy in insulin secretion with increasing feeding frequency. A reduction of 54 ± 10% in insulin AUC was seen after sipping a glucose solution over 180 min compared with consuming the same amount of glucose as a bolus (Jenkins et al., 1990).

One of the factors contributing to the reduced insulin secretion found with smaller meal sizes, may be a reduced secretion of insulin-stimulating gut hormones (e.g. cholecystokinin or glucagon-like peptide-1), which is for example shown in human (Jenkins et al., 1990; 1992), either or not in combination with a reduced rate of stomach emptying.

We conclude that increasing the feeding frequency, without increasing the total daily feed intake, is not a proper way to stimulate total insulin secretion and to keep insulin at a more constant level during the day.

IGF-1 levels were comparable between treatments, which might indicate that IGF-1 secretion is not influenced by the pattern of insulin secretion in anabolic sows. The numerically lower IGF-1 levels at d4 and 5 after weaning in CTRL sows with a low DMI_{WOI} (< 75%) compared with DL sows with a low DMI_{WOI}, are most probably related to the significantly lower DMI_{WOI} in these CTRL sows compared with the DL sows (27 vs. 50%, respectively).

This study shows that the CTRL diet fed at 12h intervals had a beneficial effect on follicle development and LH secretion compared with the DL diet fed at 4h intervals; CTRL sows had a higher pre-ovulatory LH surge and improved follicle development (larger and more uniform follicles) at d4 after weaning compared with the DL sows. The difference between number of follicles (≥ 3 mm) counted on d4 after weaning and ovulation rate was only -0.8±0.4 (ranging from -4 until 3), and therefore on average the follicle pool at d4 after weaning represents the pre-ovulatory follicle pool.
The positive effects of the CTRL diet fed at 12h intervals on follicle development and LH secretion compared with the DL diet might be related to the secretion pattern of insulin. We hypothesized before this experiment that more prolonged enhanced insulin levels (e.g. mediated through an increased feeding frequency) would be beneficial for follicle development. In this study, DL sows reached peak insulin levels more frequently (6x/d), but insulin levels rapidly dropped thereafter, whereas in CTRL sows peak levels were only reached twice a day, and insulin levels decreased only gradually thereafter. Cox et al. (1987) found no differences in LH secretion and ovulation rate between gilts injected either 4 times daily (at 6h intervals) with short-acting insulin or once daily with long-acting insulin, from d1 after altrenogest until 24h after estrus (follicular phase). However, in their study, absolute peak insulin levels were higher in gilts injected more frequently with the short-acting insulin, and the total daily amount of insulin output (AUC) was not calculated. The effect of different insulin secretion patterns on follicle development and uniformity is not studied before. Our results suggest that not only the total insulin secretion plays a role in follicle stimulation, but also the pattern of insulin secretion during the day affects this process.

Average diameter of the 5 largest follicles at d4 after weaning and at ovulation, and the percentage of follicles ≥ 7 mm at d4 did not differ between treatments, which may indicate that especially the relatively less developed follicles were extra stimulated by the feeding regimen. Indeed at d4, CTRL sows had significantly fewer small follicles (< 5 mm and < 6 mm) compared with DL sows. Insulin and IGF-1 can stimulate follicle and oocyte development via stimulation of LH (at brain level), but also directly at the ovarian level. Insulin and IGF-1 receptors are present at granulosa cells, indicating that insulin and IGF-1 can have (non-specific) growth and cell differentiation-promoting effects on ovarian cells (e.g. through general effects on glucose and amino acid metabolism). Furthermore, specific effects of insulin and IGF-1 on ovarian steroidogenesis and aromatase activity have been reported (e.g.

as reviewed by Poretsky & Kalin (1987) and Pettigrew & Tokach (1993)) and insulin increases the number of LH receptors on granulosa cells (Poretsky and Kalin, 1987). These direct actions of insulin and IGF-1 on follicle development could explain why especially the relatively less developed follicles benefit from enhanced insulin and IGF-1 levels. During follicle development, there is a shift in dependency from FSH (small follicles, 2-4 mm) to LH (> 4 mm) (Driancourt et al., 1995) and responsiveness to LH determines whether a follicle will continue growing and ovulate or will go into atresia. By increasing the number of LH receptors on granulosa cells, insulin and IGF-1 can specifically stimulate follicles of intermediate development, which still have the potential for either ovulation or atresia. Because the less developed follicles probably will not be selected and will go into atresia, the intermediate follicles that will be selected will become the less developed follicles in the pre-ovulatory follicle pool. Increasing responsiveness to LH of these intermediate follicles will rescue them from atresia (which will result in a higher ovulation rate) and can further stimulate their development (which will result in improved uniformity). This is confirmed by results of Matamoros et al. (1990) who showed that exogenous insulin injections reduced atresia in medium-sized follicles (4-6 mm) during d17 and 19 of the estrous cycle in gilts, whereas the number and atresia of small (< 3 mm) and large (≥ 7 mm) follicles was unaffected by the insulin treatment. Furthermore, Cox (1997) and Monget and Martin (1997) already reviewed that the direct ovarian action of the metabolic hormones insulin and IGF-1 is targeted mainly on medium-sized follicles.

The observation that both average follicle diameter and follicle uniformity were improved in the CTRL treatment, is in accordance with our hypothesis that especially relatively less developed follicles can benefit from extra stimulation by insulin and IGF-1 secretion. In this context, also a higher ovulation rate in CTRL sows would be expected, which was indeed the case, although not significant (ovulation rate was 24.3 vs. 23.2 in CTRL and DL sows, respectively). Results of Van den Brand et al. (2009), who found that dextrose plus lactose in the diet (when fed 2x/d) of sows during lactation and WEI resulted in more piglets born alive in the subsequent litter (difference was 0.51 piglet born alive), together with higher birth weights (difference was 86 g) and more uniform birth weights (difference of birth weight CV was 2.7%) compared with a control diet, further confirm our hypothesis.

Besides the insulin effects of the 2 dietary treatments on the hypothalamus-pituitary-ovarian axis, effects can also be mediated by corresponding changes in other metabolites or metabolic hormones (e.g. glucose, leptin, free fatty acids) related to feed composition and feeding frequency. This needs further study.

IMPLICATIONS

Absolute insulin and IGF-1 levels during the WOI showed relationships with LH secretion and follicle development in multiparous sows. Insulin secretion cannot be stimulated by an

increased feeding frequency unless the total daily feed intake is increased or specific insulin-stimulating feed components are added.

Feeding strategies (feeding frequency and diet composition) which modulate the pattern of insulin secretion, even without affecting total daily insulin output, mean insulin levels, peak insulin levels or IGF-1 levels, can influence LH secretion and development and uniformity of pre-ovulatory follicles. Our results suggest that 2 sustained insulin peaks per day (i.e. twice a day feeding) are more beneficial for follicle development and uniformity than frequent short insulin peaks per day (i.e. frequent feeding).

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Table 1. Composition of the experimental diets (as fed)

Ingredient	**DL, g**		**CTRL, g**	
Wheat	159.6		159.6	
Barley	184.1		184.1	
Palm kernel expeller (CF > 220 g/kg)	92.1		92.1	
Sugarbeet pulp (sugar < 100 g/kg)	115.0		115.0	
Wheat middlings	147.3		147.3	
Soybean meal, extracted (CF < 50 g/kg)	105.0		105.0	
Soybean hulls (CF 320-360 g/kg)	33.2		33.2	
Sugarcane molasses (sugar > 475 g/kg)	46.0		46.0	
Vitamin-mineral premix	4.6		4.6	
Limestone	6.9		6.9	
Monocalciumphosphate	5.5		5.5	
Salt	2.8		2.8	
Soybean oil	18.4		45.7	
Dextrose	39.6		-	
Lactose	39.6		-	
Total, g[1]	**1,000**		**947**	
Content	Calculated	Analyzed	Calculated	Analyzed
	g / 1,000 g		g / 947 g	
Dry matter	882.1	874.8	832.6	821.6
Crude fat	40.3	39.4	67.4	64.9
Crude protein	136.0	140.4	135.9	137.4
Starch	225.7	222.6	225.6	220.4
Glucose	136.9	113.0	58.8	59.2
kJ NE (for swine)[2]	8,860	-	8,811	-

[1] 1,000 g of the DL-diet and 947 g of the CTRL diet are isocaloric and isonitrogenic.

[2] According to the Centraal Veevoederbureau (CVB, 2003).

Table 2. Glucose and insulin parameters (means ± SE) of sows with a DMI of more than 75% at the 0800h feedings of d2 and 3, for sows fed either a dextrose and lactose- (each 150 g/d) containing diet (DL) at 4h intervals or an isocaloric control diet (CTRL) at 12h intervals during the WOI (from weaning until 12h after ovulation)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	Sampling day[2]
Number of sows	8	9		
<u>Glucose</u>				
Basal glucose, mg/dl	86.0 ± 1.6	85.8 ± 1.6	0.25	-
Glucose AUC240, mg	-1,382 ± 365	317 ± 345	< 0.001	-
Glucose AUC720, mg	-946 ± 1,362	950 ± 1,034	0.07	-
Mean glucose, mg/dl[3]	84.7 ± 1.6	86.7 ± 1.4	0.29	< 0.01
<u>Insulin</u>				
Basal insulin, µU/ml	17.3 ± 1.2	16.1 ± 1.3	0.59	-
Maximal insulin, µU/ml	74.6 ± 5.9	65.7 ± 7.9	0.48	-
Insulin increase after feeding, µU/ml	57.3 ± 5.8	49.7 ± 7.4	0.51	-
Insulin AUC240, µU	5,021 ± 676	2,856 ± 374	0.03	-
Insulin AUC720, µU	7,596 ± 1,190	8,569 ± 1,122	0.64	-
Mean insulin, µU/ml	27.8 ± 1.6	27.9 ± 2.1	0.98	-

[1] Statistical significance; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).
[2] Day 2 or 3 after weaning (sampling days for determination of glucose and insulin profiles).
[3] Treatment*sampling day interaction ($P < 0.01$) (LSmeans CTRL d2 = 80.1^{a} mg/dl, CTRL d3 = 89.3^{b} mg/dl, DL d2 = 87.6^{b} mg/dl, DL d3 = 86.8^{b} mg/dl).

Table 3. Follicle development, estrus and ovulation, and LH characteristics (means ± SE) for sows fed either a dextrose and lactose- (each 150 g/d) containing diet (DL) at 4h intervals or an isocaloric control diet (CTRL) at 12h intervals during the WOI (from weaning until 12h after ovulation)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	DMI_{WOI}[2]
Number of sows	15[4]	16[4]		
<u>Follicle development</u>				
Follicle diameter at weaning, mm[3]	3.3 ± 0.2	3.4 ± 0.1	0.81	n.a.
Follicle diameter at d4 after weaning, mm[3,4]	6.8 ± 0.2	6.8 ± 0.1	0.90	-
Follicle diameter at ovulation, mm[3,4]	7.0 ± 0.2	6.9 ± 0.1	0.58	-
<u>Estrus and ovulation</u>				
Weaning-to-estrus interval, h[4]	97 ± 3.1	95 ± 3.1	0.67	-
Estrus duration, h[4]	57 ± 2.8	54 ± 3.1	0.18	-
Weaning-to-ovulation interval, h	140 ± 3.1	136 ± 3.0	0.40	-
Ovulation rate	24.3 ± 1.2	23.2 ± 0.8	0.43	-
<u>LH</u>				
Basal LH, ng/ml	0.96 ± 0.05	0.92 ± 0.05	0.99	-
LH surge level, ng/ml	3.73 ± 0.24	3.00 ± 0.18	0.03	0.06[5]
Interval weaning to LH surge, h	107 ± 3.1	102 ± 2.5	0.22	-

[1] Statistical significance; the treatment*DMI_{WOI} interactions were not significant; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).

[2] DMI from weaning until 12h after ovulation, as % of total dry matter offered (< 75%, ≥ 75%); n.a. = not applicable.

[3] Average diameter of 5 largest follicles at one ovary.

[4] Additionally, 1 DL sow developed cystic ovaries (OR: 3; WEI: 120h), 1 DL sow had a silent estrus, and for 3 sows no ultrasound clips at d4 were available.

[5] LSmeans were 3.6 and 3.1 ng/ml for sows with a low (< 75%) and high (≥ 75%) DMI_{WOI}, respectively.

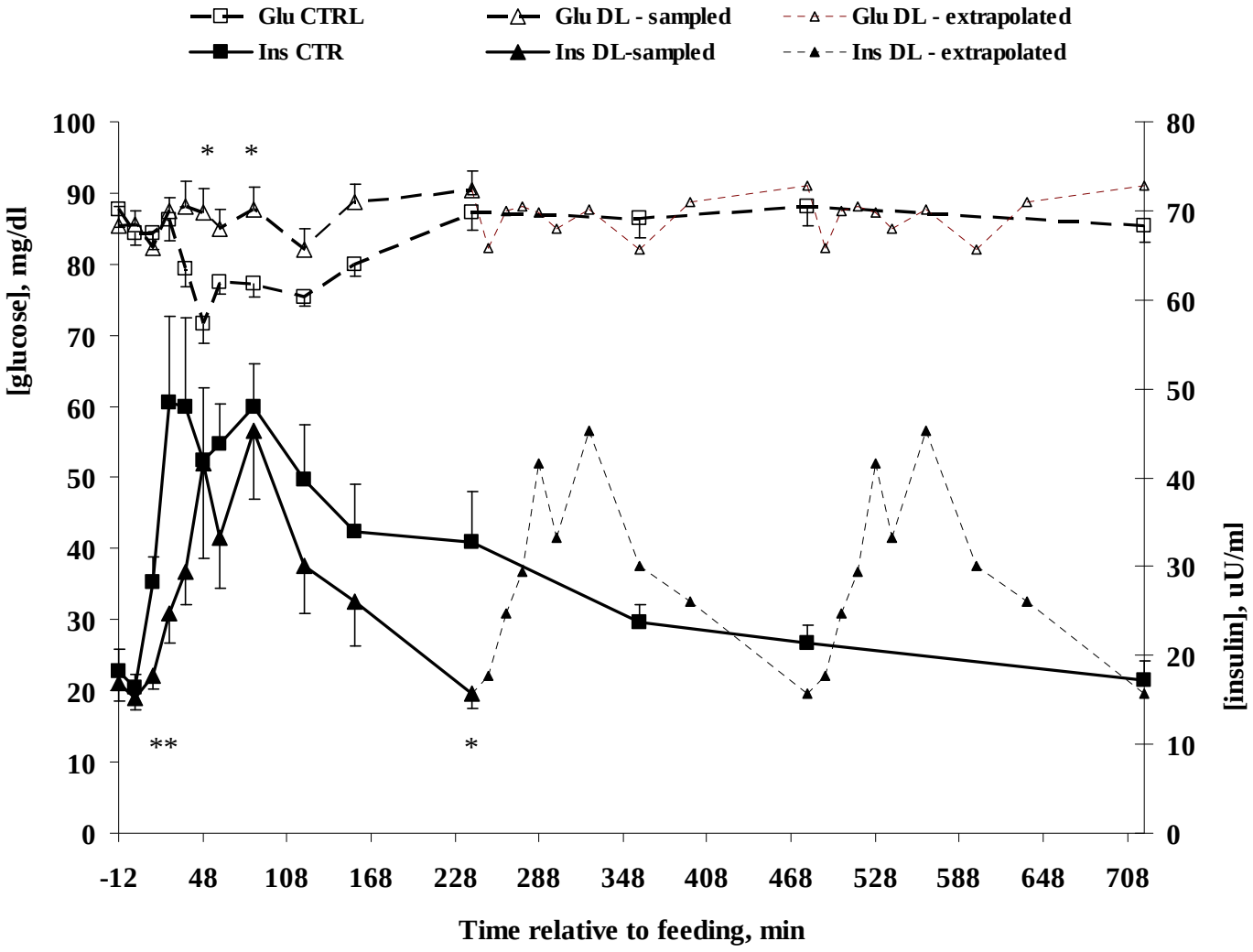

Figure 1. Glucose and insulin profiles (means±SE) around 0800h feeding of sows with DMI of ≥75% at d2 and 3 after weaning, fed either a dextrose and lactose- (each 150 g/d) containing diet at 4h intervals (DL; n = 18 sow days; sampled from t = -12 until t = 240 min, extrapolated thereafter because sows were fed again at t = 240 and t = 480 min), or an isocaloric control diet at 12h intervals (CTRL; n = 16 sow days; sampled from t = -12 until t = 720 min) during the WOI; * = CTRL vs. DL, $P < 0.05$

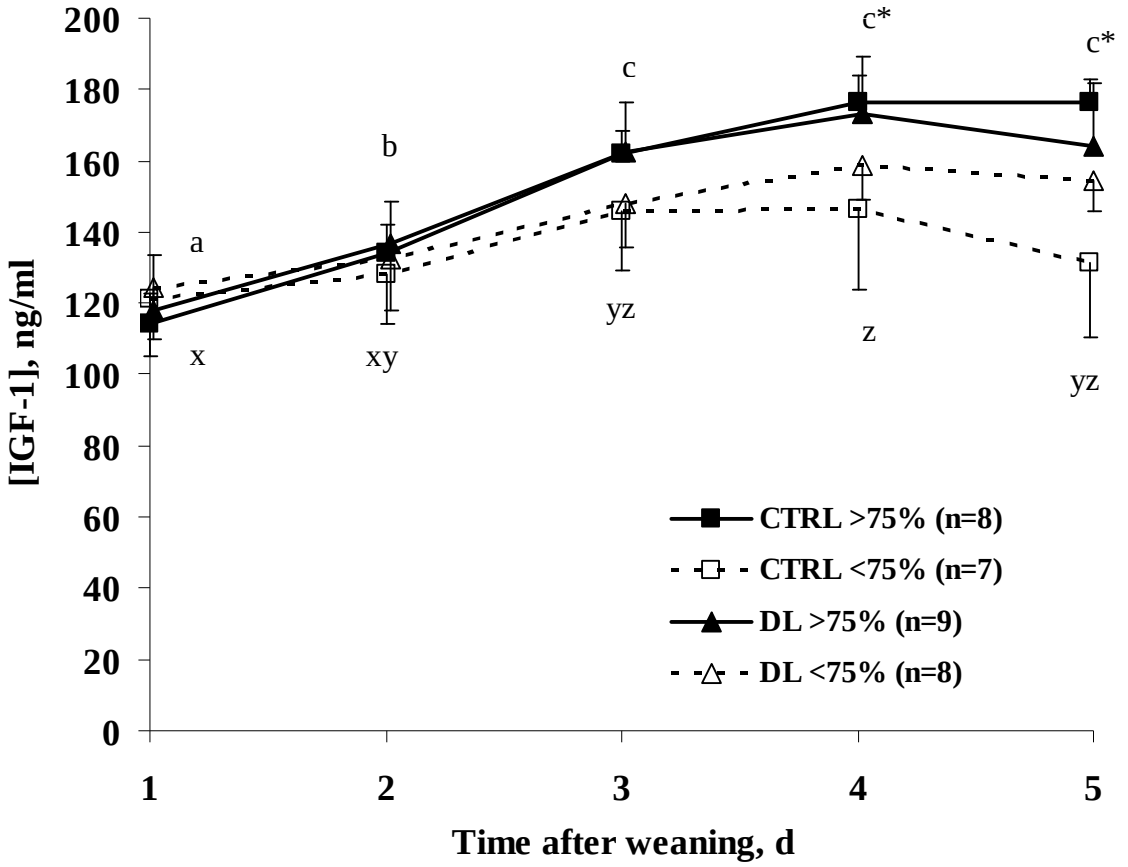

Figure 2. IGF-1 profiles (means±SE) from weaning until d5 after weaning of sows with a high DMI_{WOI} (≥ 75%; as % of total dry matter offered from weaning until 12h after ovulation) or low DMI_{WOI} (< 75%), for sows fed either a dextrose and lactose- (each 150 g/d) containing diet at 4h intervals (DL), or an isocaloric control diet at 12h intervals (CTRL) during the WOI (from weaning until 12h after ovulation); * effect DMI_{WOI}, $P < 0.05$; abc for sows with high DMI_{WOI} (≥ 75%), days with different superscript differ, $P < 0.05$; xyz for sows with low DMI_{WOI} (< 75%), days with different superscript differ, $P < 0.05$

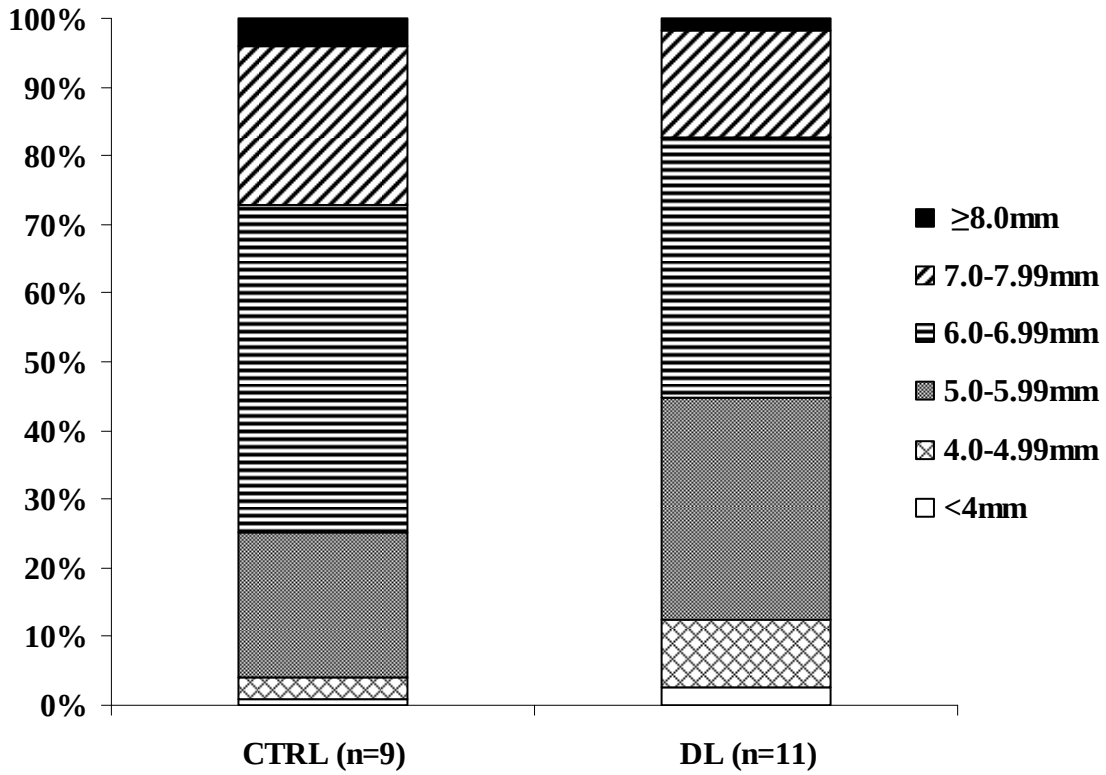

Figure 3. Percentage of follicles (means) in each diameter category at d4 after weaning, for sows fed either a dextrose and lactose- (each 150 g/d) containing diet (DL) at 4h intervals or an isocaloric control diet (CTRL) at 12h intervals during the WOI (from weaning until 12h after ovulation)

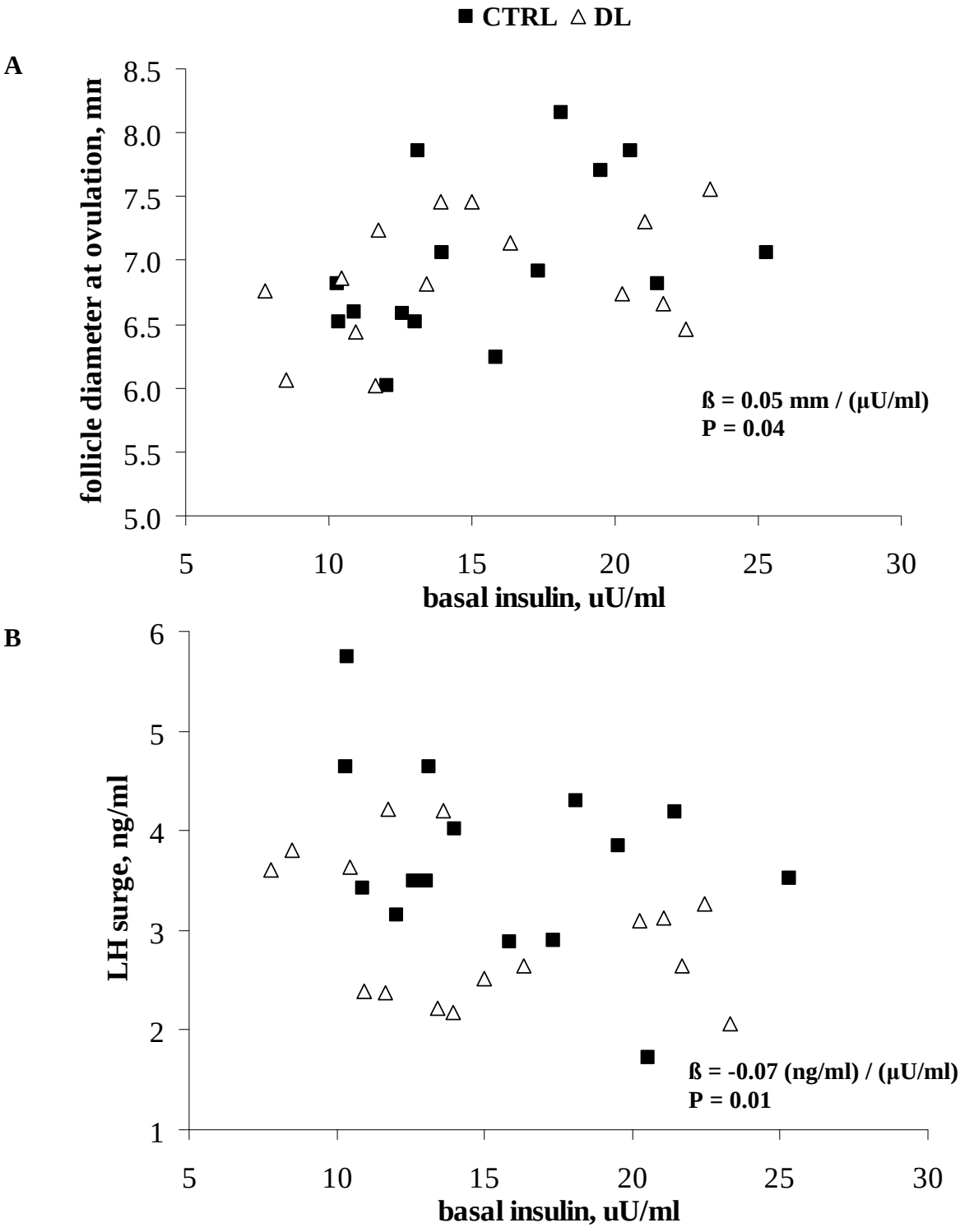

Figure 4. Relations between basal insulin level (mean d2 and 3 after weaning) with A). follicle diameter at ovulation; and B). LH surge level (absolute LH surge level was significantly higher in CTRL compared with DL; 3.73 vs. 3.00 ng/ml; *P* = 0.03)

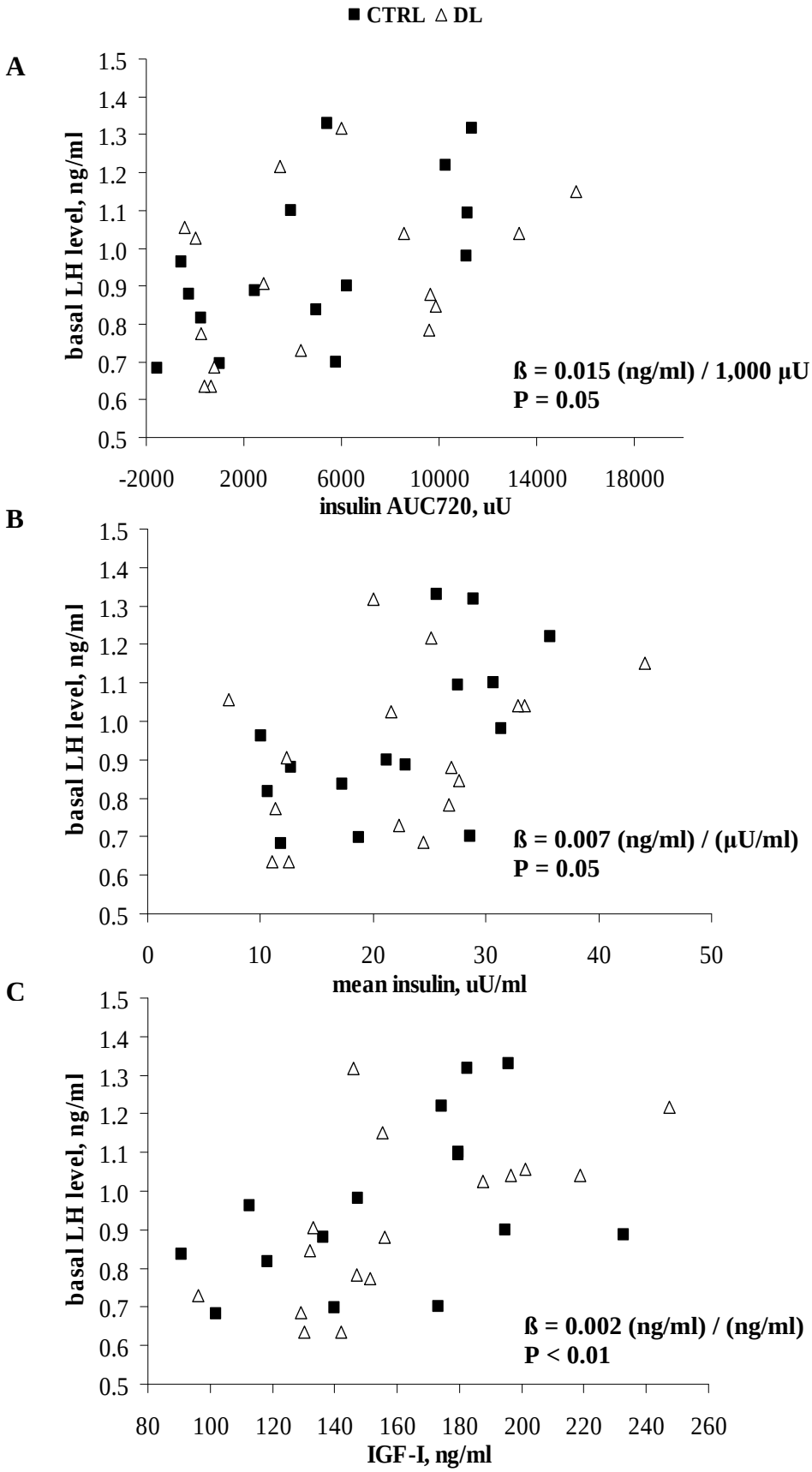

Figure 5. Relations between A). insulin AUC720 (mean d2 and 3 after weaning); B). mean insulin (mean d2 and 3 after weaning); and C). IGF-1 (mean level during d3-5 after weaning) with basal LH level

BIJLAGE C. PAPER EXPERIMENT 1 - DEEL II

Running head: Conceptus development and uniformity in sows

Title: Diet composition and feeding frequency during the weaning-to-ovulation interval in multiparous sows: II. Effects on luteal development, progesterone and conceptus development and uniformity[1]

Authors: J.G.M. Wientjes[2], N.M. Soede, B.F.A. Laurenssen, R.E. Koopmanschap, H. van den Brand and B. Kemp

Adaptation Physiology Group, Wageningen University, P.O. Box 338, 6700AH, Wageningen, The Netherlands

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[2] To whom correspondence should be addressed: P.O. Box 338, 6700AH (phone: +31 317 486328; fax: +31 317 485006; E-mail: anne.wientjes@wur.nl).

ABSTRACT: Dextrose and lactose in the pre-mating sow diet improve litter uniformity, possibly related to an insulin and IGF-1 stimulating effect. We studied effects of nutritionally induced differences in insulin during the weaning-to-ovulation interval (WOI) on luteal development, progesterone secretion and pre-implantation conceptus development and uniformity. To create insulin contrasts, 32 multiparous sows were fed either a dextrose plus lactose containing diet (DL; each 150 g/d) at 4h intervals or a control diet (CTRL; containing soybean oil) fed isocalorically and isonitrogenously at 12h intervals during the WOI. After ovulation, all sows received a standard gestation diet at 12h intervals. At d10 of pregnancy sows were slaughtered to asses luteal and conceptus development and uniformity. Ovulation rate, plasma progesterone levels, pregnancy rate and embryo survival rate did not differ between treatments. CTRL sows had a higher total luteal weight (mean corpus luteum diameter: 10.0 vs. 9.6 mm, $P = 0.06$; mean corpus luteum weight: 0.47 vs. 0.42 g, $P = 0.09$; total luteal weight: 11.2 vs. 9.7 g, $P = 0.03$) and mean corpus luteum diameter and weight were positively correlated with mean follicle diameter of all follicles ≥ 3 mm at d4 after weaning ($r \geq 0.55$; $P \leq 0.02$). CTRL sows tended to have larger conceptuses at d10 of pregnancy (average diameter: 7.1 vs. 6.4 mm; $P = 0.07$). Conceptus uniformity was not influenced by treatment. Insulin AUC and mean insulin (mean values of d2 and 3 after weaning) were positively related with mean progesterone ($P < 0.05$) and maximal progesterone ($P < 0.05$) levels during the first 10d of pregnancy. Insulin AUC (mean value of d2 and 3 after weaning) was also positively related with conceptus diameter ($P = 0.03$). Insulin and IGF-1 levels during the WOI were not related to conceptus uniformity. From the present study, it can be concluded that high insulin levels during the WOI are beneficial for luteal and conceptus development in sows. Those effects are most probably mediated through beneficial effects of insulin on follicle and oocyte development. The higher progesterone levels and more developed conceptuses might be beneficial for development and uniformity of fetuses and piglets at later stages of pregnancy.

Key words: Conceptus development, conceptus uniformity, insulin, nutrition, progesterone, sows

INTRODUCTION

The increased litter size over the last decades is associated with increased pre-weaning piglet mortality, which is partly related with decreased litter uniformity (Milligan et al., 2002). Quesnel et al. (2008) concluded that sow factors as litter size, parity and season at conception together explained 20% of litter uniformity at birth, which indicates that a major part of the variation in litter uniformity is caused by other yet unknown factors.

Recent studies suggest that nutrition of the sow in the pre-mating period can influence uniformity in piglet development. Fermentable NSP (50% sugarbeet pulp) in the pre-mating gilt diet improved uniformity in fetal weights at d27 of gestation (Ferguson et al., 2006), and

dextrose (150 g/d), either or not in combination with lactose (150 g/d), in the pre-mating sow diet improved litter uniformity at birth (Van den Brand et al., 2006; 2009). The physiological mechanisms involved are unknown, but insulin and IGF-1 seem to be potential mediators. Dextrose in the diet of gilts and lactating sows resulted in a faster, higher and longer insulin peak after feeding and increased IGF-1 levels compared to fat-rich diets (Van den Brand et al., 1998; 2001; Zieçik et al., 2002) and fermentable NSP enhance insulin levels for a prolonged period (Vestergaard, 1997). Insulin and IGF-1 are known to stimulate follicle and oocyte development, either indirectly at the brain level via stimulation of LH (Koketsu et al., 1996; Van den Brand et al., 2001), or directly at the ovarian level (Poretsky and Kalin, 1987; Quesnel et al., 2007; 2009).

As a first step to unravel the mechanism behind the effect of pre-mating sow diets on litter uniformity, we studied the effect of nutritionally induced differences in insulin secretion patterns, and corresponding IGF-1 levels, during the weaning-to-ovulation interval (WOI) on luteal development, progesterone secretion and embryo development and uniformity at d10 of gestation.

MATERIALS AND METHODS

General Design

During the WOI, multiparous (parity 5.9±0.3; range 3-9) Topigs 20 (Topigs, Vught, the Netherlands) sows (n = 38) were fed either a dextrose plus lactose-containing diet (DL) at 4h intervals or an isocaloric and isonitrogenous control diet (CTRL; dextrose and lactose was exchanged by soybean oil) at 12h intervals (0800h and 2000h) from day of weaning until ovulation. After ovulation, all sows received a standard gestation diet at 12h intervals (0800h and 2000h) until slaughter at d10 after ovulation.

Sows arrived at the experimental farm of Wageningen University within 2h from weaning (d0), in 3 consecutive batches. At day of weaning, sows received either a permanent jugular vein catheter (23 sows) for blood sampling during the whole experimental period, or an ear vein catheter (15 sows) for blood sampling during the WOI. From d2 after weaning, estrus detection was performed at 4h intervals by a back-pressure test in the presence of a vasectomized boar. To determine time of ovulation, sows were transrectally scanned (Scanner 200, Pie Medical/Esaote, Maastricht, the Netherlands) at 12h intervals (0800h and 2000h) from 12h after the onset of estrus. Sows were inseminated every day of estrus with a commercial dose of semen (containing 2×10^9 sperm cells) of a Topigs boar line, until ovulation had occurred.

Six sows (4 CTRL, 2 DL) were veterinary treated for pneumonia (5 sows) or diarrhea (1 sow) during the experiment and were excluded from all analyses. All experimental procedures were

approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, the Netherlands).

A complete description of animals used, animal management and diet compositions are reported elsewhere (Wientjes et al., submitted).

Data on insulin and IGF-1 profiles during WOI, follicle development, estrus and ovulation are reported elsewhere (Wientjes et al., submitted). This paper focuses on effects on luteal development, progesterone, and conceptus development and uniformity.

Blood Sampling

Frequent blood samples were taken around the 0800h feeding at d2 and 3 after weaning for determination of glucose and insulin, and 0800h samples at d1, 2, 3, 4 and 5 after weaning were analyzed for IGF-1, as described elsewhere (Wientjes et al., submitted). From d1 after weaning (0800h) until time of ovulation, blood samples were taken at 4h intervals for all sows. Only for sows with a jugular vein catheter, plasma samples were taken after ovulation. Plasma samples taken at 4h intervals from 6h before until 18h after time of ovulation, and at 12h intervals thereafter until slaughter were analyzed for progesterone. For sows with an ear vein catheter, one blood sample was taken at slaughter at d10 of pregnancy for determination of progesterone. Furthermore, the 0800h samples taken at d1, 3, 7 and 10 after ovulation were analyzed for IGF-1.

Blood samples were collected in polypropylene tubes containing 100 µl EDTA solution (144 mg/mL saline; Tritiplex III, Merck Nederland B.V., Amsterdam, the Netherlands), immediately placed on ice after collection and centrifuged at 1,710 x *g* for 10 min at 4 ºC. Plasma was stored at - 20 ºC until analyses.

Uteri, Ovaries and Conceptuses

Sows were slaughtered at the experimental farm 9.5 or 10d after ovulation, depending on time of ovulation. Sows that had ovulated at 2000h were slaughtered 9.5d later; sows that had ovulated at 0800h were slaughtered 10d later. Immediately after stunning and exsanguination, reproductive tracts were removed and placed on ice until further processing at the laboratory within 1h.

At the laboratory, uteri and cervix were separated from the ovaries, oviducts and mesometrium. Both uterine horns were flushed twice with 30 ml 0.9% NaCl from the cervical to the ovarian end to collect the conceptuses. Uterine fluids of each uterine horn (first flushing only) were centrifuged at 4,500 x *g* for 15 min at 4 ºC and supernatant was stored at - 20 ºC until analyses. Conceptuses were immediately placed in 30 mL Dulbecco's PBS and kept on ice. The largest diameter (di1) and the largest diameter perpendicular to it (di2) of each conceptus (magnification of 1x) and its embryoblast (magnification of 6.3x) were measured using a stereo-microscope after spreading the conceptus as much as possible. Conceptuses were stored in 200 µL distilled water at - 20 ºC, thawed and frozen 5 times and sonificated (2 x 10 pulses

at 30% of maximal energy; Branson Sonifier 250, Boom BV, Meppel, The Netherlands) until further analyses.

For diameter analyses the largest diameter (di1) was used. Conceptus surface area was calculated as: $2\pi ab$, where $a = {}^1/_2$ di1; and $b = {}^1/_2$ di2. When no conceptuses were recovered, sows were considered non-pregnant (n = 3, including one sow (DL) that developed cystic ovaries). Additionally, in one sow (DL) with turbid uterine flushings, all conceptuses (n = 10) were non-vital, based on abnormal morphological appearance (abnormal dark/brown color and shriveled up). Other parameters (ovulation rate, luteal development and progesterone levels) seemed normal, and therefore we considered this sow as pregnant at d10 of pregnancy. Furthermore, 1 sow (DL) was not inseminated due to silent estrus.

Number of corpora lutea (CL) was counted and total ovary weight was measured. Individual CL's were dissected and diameter, weight and quality (normal, hemorrhagic, cystic) was determined. Total luteal weight was calculated as the sum of weights of the individual CL's. Embryo survival was defined as the number of conceptuses divided by the number of CL's.

Plasma Analyses

Analysis of plasma glucose, insulin and IGF-1 levels, and a complete definition of glucose, insulin and IGF-1 parameters used, are described elsewhere (Wientjes et al., submitted). For each sampling day, basal insulin levels were calculated as the mean value of the 2 samples taken before feeding (- 12 and 0 min); AUC720 (area under the curve during a 720 min period) was calculated as the area above basal insulin levels from feeding until 720 min after feeding for CTRL sows, and as AUC during 4h multiplied by 3 for DL sows as DL sows were fed again at 1200h and 1600h; and the mean insulin level during the sampling period was calculated as the average insulin levels of all plasma samples after feeding (from 0 until 720 min in CTRL; from 0 until 240 min in DL) corrected for the time intervals between samples.

Progesterone levels were determined in duplicate, using a commercial Coat-A-Count Progesterone RIA-kit (PITKPG-7, Siemens Medical Solutions Diagnostics, Los Angeles, USA). The sensitivity, intra- and interassay CV were 0.1 ng/ml, 4.7% and 6.0%, respectively. Basal progesterone levels were calculated as the average value of the first 2 samples (6h and 2h before ovulation); maximal progesterone levels were calculated as the average value of the last 2 samples before slaughter. Mean progesterone levels were calculated as the average progesterone level of all plasma samples, corrected for the time intervals between samples. For sows with an ear vein catheter, maximal progesterone level was defined as progesterone level at slaughter.

Conceptus analyses

Protein content of conceptuses was analyzed in duplicate with the method as described by Bradford (1976), using the microplate-procedure of a commercial kit (QuickStart Bradford Protein Assay, Bio-Rad, Hercules, USA), with Bovine Serum Albumin as standard. DNA

content of conceptuses was measured fluorometrically in duplicate using a commercial kit (Quant-iT dsDNA Assay Kit, Broad Range Q33130; Invitrogen, Ltd, Paisley, UK).

Uterine fluids analyses

Uterine fluids were analyzed on acid phospatase activity, calcium, glucose, insulin and IGF-1 content. Acid phosphatase activity was determined photometrically in triplicate with 4-nitrophenylphosphate as substrate using the method of Bergmeyer H.U. (1974; Methoden der enzymatischen analyse, Band I, 3. Auflage, Weinheim). One unit of activity was defined as the capacity to release 1 μmol nitrophenol per min at pH = 4.8 in substrate buffer (50 mM citrate, 5.5 mM 4-nitrophenylphosphate) at 25 ºC.

Calcium levels were determined photometrically in duplicate with the cresolphthalein complexone (CPC) method using a commercial kit (Calcium CPCFS, DiaSys Diagnostic Systems GmbH, Holzheim, Germany), with a lower detection limit of 0.2 mg/dL. Glucose, insulin and IGF-1 were analyzed as described for plasma analyses (see Wientjes et al., submitted), but glucose and IGF-1 were not detectable in uterine fluids.

Statistical Analyses

Data are presented as means±SE, unless otherwise stated. Data were analyzed with the GLM procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC), unless otherwise stated. For all GLM analyses, first interaction terms were tested and removed from the model when not significant ($P > 0.10$), followed by a stepwise removal of the variable with the highest non-significant P-value ($P > 0.10$), except for the factor treatment.

Analysis of insulin and IGF-1 levels during WOI is described elsewhere (Wientjes et al., submitted).

IGF-1 levels during pregnancy. IGF-1 levels during early pregnancy were analyzed using model 1: $Y_{ijklmn} = \mu + T_i + B_j + DMI_{WOI\_k} + e1_{ijkl} + SD_m + T_i*SD_m + T_i*DMI_{WOI\_k} + SD_m*DMI_{WOI\_k} + T_i*SD_m*DMI_{WOI\_k} + e2_{ijklmn}$, where Y_{ijklmn} = dependent variable; μ = overall mean; T_i = treatment (i = CTRL, DL); B_j = batch (j = 1,2,3); DMI_{WOI_k} = class of DMI from weaning until 12h after ovulation (dietary treatment period), as % of total dry matter offered (k = 0 (< 75%), 1 (≥ 75%)); $e1_{ijkl}$ = error term 1, which represents the random effect of sow_l (l = 1 to 32) nested within treatment, batch and DMI_{WOI}; SD_m = sampling day (m = d1,3,7,10 after ovulation); T_i*SD_m = interaction between treatment and sampling day; T_i* DMI_{WOI_k} = interaction between treatment and DMI_{WOI}; SD_m* DMI_{WOI_k} = interaction between sampling day and DMI_{WOI}; $T_i*SD_m*DMI_{WOI\_k}$ = interaction between treatment, sampling day and DMI_{WOI}; and $e2_{ijklm}$ = residual error. The effect of treatment, batch and DMI_{WOI} was tested against error term 1. Effects of sampling day and all interactions were tested against the residual error.

Luteal development and progesterone. One sow (DL) developed cystic ovaries (weaning-to-estrus interval: 120h; ovulation rate: 3; no conceptuses recovered) and was excluded from all

analyses on luteal development and progesterone. Luteal development and plasma progesterone levels of remaining non-pregnant sows (n = 3) and the sow with non-vital embryos seemed normal and were therefore included in all analyses. Two sows (both CTRL) had cystic CL's on both ovaries (10 of 22 and 4 of 24 CL's) and were excluded from analyses on total luteal weight, CL diameter and CL weight.

Ovulation rate, luteal development (total luteal weight, mean CL diameter, mean CL weight) and uniformity (SD and CV of mean CL diameter and weight), and progesterone traits (basal, mean and maximal) were analyzed using model 2: $Y_{ijkl} = \mu + T_i + B_j + DMI_{WOI\_k} + T_i*DMI_{WOI\_k} + e_{ijkl}$, where Y_{ijkl} = dependent variable; μ = overall mean; T_i = treatment (i = CTRL, DL); B_j = batch (j = 1,2,3); DMI_{WOI_k} = DMI from weaning until 12h after ovulation (dietary treatment period), as % of total dry matter offered (k = 0 (< 75%), 1 (≥ 75%)); $T_i*DMI_{WOI\_k}$ = interaction between treatment and DMI_{WOI_k}; and e_{ijkl} = random error.

To check whether differences in luteal development could be explained by differences in pre-ovulatory follicle development (as described elsewhere; Wientjes et al., submitted), Pearson correlation coefficients were calculated between luteal development (mean CL diameter and weight) and follicle diameter (average diameter of largest 5 follicles at one ovary at d4 after weaning and at ovulation for all sows; average diameter of all follicles ≥ 3 mm at d4 after weaning for 20 sows with complete ultrasound clips) parameters, and follicle diameter parameters were added as a covariable to model 2. When luteal characteristics were significantly affected by batch, batch-corrected residuals were used for calculation of correlations.

Conceptus development and uniformity. Differences in numbers of sows being pregnant between treatments were analyzed using a logistic model (using the logistic procedure) with pregnant (0/1; excluding the sow not inseminated due to silent estrus) as outcome variable and treatment as the only factor. Non-pregnant sows (n = 3), 1 sow with non-vital embryos, and 1 sow not inseminated, were excluded from analyses on number of conceptuses, embryo survival and conceptus development and uniformity. Number of conceptuses and embryo survival rate were analyzed using model 2. For conceptus characteristics (diameter, surface area, embryoblast diameter, protein content and DNA content) and uniformity (SD and CV of diameter, surface area, protein content and DNA content), age of the conceptuses (9.5 or 10d) was added as a factor to model 2. Two sows (1 CTRL and 1 DL) contained filamentous conceptuses and were excluded from all analyses on conceptus development and uniformity.

To check whether differences in conceptus development could be explained by differences in pre-ovulatory follicle development (as described elsewhere; Wientjes et al., submitted), Pearson correlation coefficients were calculated between conceptus development (conceptus diameter and conceptus protein) and follicle diameter (average diameter of largest 5 follicles at one ovary at d4 after weaning and at ovulation for all sows; average diameter of all follicles ≥ 3 mm at d4 after weaning for 20 sows with complete ultrasound clips) parameters, and

follicle diameter parameters were added as a covariable to model 2. When conceptus characteristics were significantly affected by batch and age of the conceptuses, batch- and conceptus age-corrected residuals were used for calculation of correlations.

Uterine secretions. Uterine secretions (insulin, calcium and acid phosphatase activity) per horn were calculated by multiplying the concentrations (insulin, calcium and the acid phosphatase activity) with the flushing volume (30 ml). Average uterine secretions were analyzed at sow level, by averaging the concentrations of both horns per sow, with the same model as used for conceptus characteristics (model 2 with addition of age of the conceptuses (9.5 or 10d) as factor to the model). Non-pregnant sows (n = 3), 1 sow with non-vital embryos, 1 sow not inseminated, and sows with filamentous conceptuses (n = 2) were excluded from analyses on uterine secretions.

Relations with insulin and IGF-1. Relations between insulin and IGF-1 during WOI with all reproductive parameters were analyzed by adding insulin parameters (basal insulin, insulin AUC720 or mean insulin; all mean values of both sampling days (d2 and 3) per sow or IGF-1 parameter (mean IGF-1 level during d3-5 after weaning per sow) as a covariate and its interaction with treatment to model 2. Additionally, Pearson correlation coefficients (using the CORR procedure) were calculated amongst the different insulin and IGF-1 parameters that were significantly related to reproductive parameters, and amongst the different reproductive parameters that were significantly related to insulin and IGF-1 parameters. When reproductive characteristics were significantly affected by batch and/or age of the conceptuses, batch and/or conceptus-age corrected residuals were used for calculation of correlations.

RESULTS

Dry matter intake and IGF-1 levels during pregnancy

After ovulation, all sows had a 100% DMI. IGF-1 levels after ovulation were not influenced by treatment ($P = 0.61$), nor by DMI_{WOI}. IGF-1 levels were on average 154.6 ± 8.9 ng/ml at d1, 157.0 ± 7.8 ng/ml at d3, 150.6 ± 6.5 ng/ml at d7 after ovulation, and became significantly lower at d10 after ovulation (133.8 ± 5.9 ng/ml; $P < 0.01$).

Luteal development and progesterone

Ovulation rate was not influenced by treatment, nor by DMI_{WOI} (Table 1). Total luteal weight was higher in CTRL sows (+ 1.5 g; $P = 0.03$), and mean CL diameter and mean CL weight tended to be higher in CTRL (+ 0.4 mm ($P = 0.06$) and + 0.05 g ($P = 0.09$), respectively) compared with DL sows (Table 1). Additionally, mean CL diameter was negatively affected by DMI_{WOI} (LSmeans were 10.4 and 9.4 mm for sows with a low (< 75%) and high (≥ 75%) DMI_{WOI}, respectively; $P < 0.01$).

Mean CL diameter and CL weight were not related with average diameter of the 5 largest follicles at d4 after weaning and at ovulation. However, average diameter of all follicles ≥ 3 mm present at d4 after weaning was positively correlated with CL diameter (r = 0.55; $P = 0.02$) and CL weight (r = 0.61; $P < 0.01$). After addition of average diameter of all follicles ≥ 3 mm present at d4 after weaning to the models for CL diameter and CL weight, the effect of treatment on CL diameter and CL weight was not significant anymore (P-value for treatment was 0.84 and 0.22 for CL diameter and CL weight, respectively).

Plasma progesterone levels (basal, mean and maximal) were comparable between treatments (Table 1). Mean progesterone was positively affected by DMI_{WOI} (LSmeans were 13.60 and 16.31 ng/ml for sows with a low (< 75%) and high (≥ 75%) DMI_{WOI}, respectively; $P = 0.05$).

Conceptus development and uniformity

A total of 28 sows were pregnant at slaughter and pregnancy rate did not differ between treatments (93% in CTRL; 88% in DL, $P = 0.59$). Conceptus characteristics are presented in Table 2. Number of conceptuses, embryo survival and conceptus development and uniformity were not influenced by DMI_{WOI}. Number of conceptuses and embryo survival was comparable between treatments. Conceptus diameter tended to be higher in CTRL than in DL sows (+ 0.7 mm; $P = 0.07$); conceptus surface area, embryoblast diameter, conceptus protein content and DNA content did not differ between treatments. Litter uniformity in conceptus diameter, conceptus surface area, embryoblast diameter and protein content was also comparable between treatments. Within-sow SD of conceptus DNA content tended to be lower in DL (- 31 ng; $P = 0.08$), but CV of conceptus DNA did not differ between treatments. Mean conceptus diameter and conceptus protein were not related to follicle diameter at d4 after weaning or at ovulation.

Uterine secretions

Uterine insulin (40.1 ± 4.4 μU/horn), calcium (0.41 ± 0.09 mg/horn) and acid phosphatase activity (2.98 ± 0.30 U/horn) were not influenced by treatment, nor by DMI_{WOI}. Uterine calcium and acid phosphatase activity increased with age of conceptuses (LSmeans were 0.18 and 0.57 mg/horn for calcium ($P = 0.03$) and 1.81 and 3.62 U/horn for acid phosphatase activity ($P < 0.01$) for 9.5 and 10d-old conceptuses, respectively).

Relationships between insulin and IGF-1 with reproduction parameters

No interactions existed between treatment and insulin parameters ($P > 0.10$; mean values of d2 and 3) or IGF-1 ($P > 0.10$; mean value of d3-5 after weaning), indicating that relationships were similar for both treatments, and therefore overall treatment corrected regressions are presented. An overview of all relations ($P < 0.05$) is given in Table 3.

Luteal development and progesterone. Insulin parameters were not related to ovulation rate, total luteal weight and CL weight. Mean insulin was negatively related to mean CL diameter (Table 3 & Figure 1). A positive relation existed between both insulin AUC720 and mean insulin with mean progesterone levels during the first 10d of pregnancy (Table 3 & Figure 2A), as well as with maximal progesterone levels at d10 of pregnancy (Table 3 & Figure 2B). Insulin parameters were not related to basal progesterone levels.

Mean IGF-1 during d3-5 after weaning was not related to luteal development and progesterone levels.

Conceptus development and uniformity. Insulin and IGF-1 parameters were not related to number of conceptuses and embryo survival. Basal insulin was not related to conceptus development and uniformity.

Insulin AUC720 was positively related with conceptus diameter (Table 3 & Figure 3) and SD of conceptus surface area (Table 3).

Mean IGF-1 levels at d3-5 after weaning were not related to conceptus development and conceptus uniformity.

Uterine secretions. Insulin and IGF-1 levels parameters were not related to uterine insulin, calcium and acid phosphatase activity.

Insulin AUC720 and mean insulin were highly correlated (r = 0.86; $P < 0.0001$).

Furthermore, correlations amongst the different reproductive parameters that were significantly related to one or more insulin parameters were calculated. CL diameter was not correlated with mean ($P = 0.86$) or maximal ($P = 0.44$) progesterone, but mean and maximal progesterone were highly correlated (r = 0.87; $P < 0.0001$). Conceptus diameter and SD of conceptus surface area were highly correlated (r = 0.72; $P < 0.0001$). CL diameter and mean progesterone were not correlated with the conceptus characteristics, but maximal progesterone was correlated with conceptus diameter (r = 0.44; $P = 0.03$; Figure 4).

DISCUSSION

This study was designed to investigate the mechanism behind the effect of pre-mating sow diets on litter uniformity as found in studies of Ferguson et al. (2006) and Van den Brand et al. (2006; 2009). We hypothesized that (nutritionally induced) stimulation of insulin and/or IGF-1 release during the pre-ovulatory phase can increase development and uniformity of the antral follicle pool by stimulation of the relatively less-developed follicles (via stimulation of LH and/or FSH, or directly at the ovarian level), resulting in a more uniform pre-ovulatory follicle pool (see Wientjes et al., submitted), which then results in a). a more uniform maturation of oocytes, which will be reflected in a more uniform embryo development (based on results of Xie et al. (1990) and Pope et al. (1990)) and finally more uniform birth weights within a litter (based on the observation of Van der Lende et al. (1990) that within-litter variation in

embryonic development at d35 of pregnancy is representative for the within-litter variation in piglet birth weight); and b). an improved luteal development and progesterone production.

Results of this study partly confirm our hypothesis. Positive relationships were found between pre-ovulatory insulin levels and a). LH secretion and follicle diameter (Wientjes et al., submitted); b). conceptus development at d10 of pregnancy; and c). progesterone levels during the first 10d of pregnancy. Furthermore, follicle size was reflected in size of the CL's. However, pre-ovulatory insulin and IGF-1 levels were not related to uniformity of pre-ovulatory follicles and conceptuses at d10 of pregnancy in this study**.** This could indicate that insulin does not improve uniformity of follicles and conceptuses, but stimulates the development of follicles and conceptuses in general. After d10 of pregnancy, conceptuses start to elongate and the implantation process starts. During this peri-implantation period, part of the embryos will be lost (Pope and First, 1985). Additionally, conceptus development at d10 of pregnancy is a highly variable trait, due to the rapid development of conceptuses at this stage. Therefore, it is possible that uniformity of d10 conceptuses is not a reliable predictor for piglet uniformity at birth, and it seems likely that effects of pre-mating insulin levels on piglet uniformity are not yet visible at this early stage of pregnancy.

Ferguson et al. reported already that high fibre diets prior to ovulation, which improved oocyte maturation (2007), are also beneficial for survival and uniformity of fetuses at d27 of pregnancy (2006), and result in a higher number of piglets born (11.5 vs. 10.9 piglets born alive compared with an isocaloric control diet (2004)). Van den Brand et al. (2009) showed that dextrose plus lactose in the diet prior to ovulation not only numerically improved uniformity in birth weights (- 3% in birth weight CV), but also numerically increased litter size (13.5 vs. 13.0 piglets born alive compared with an isocaloric control diet); the lack of significance in their study is probably related to the experimental setup, in which stable (6-12 sows) was used as the experimental unit. This suggests that the pre-mating diets used not only improve litter uniformity, but also improve embryonal survival. The beneficial effects of insulin on progesterone levels and conceptus development found in the current study, may therefore improve embryonal survival. Whether and how the improved progesterone production and improved conceptus development during early pregnancy lead to a more uniform development of fetuses and piglets at later stages of pregnancy needs further study.

On the other hand, the fact that we did not find relationships between insulin levels (measured at d2 and 3 after weaning) and uniformity of follicles and conceptuses could also indicate that uniformity of follicles/conceptuses is already determined earlier, e.g. around weaning or even during lactation. Ashworth et al. (1999b) fed gilts at a high feeding level during an entire estrus cycle before ovulation, and found positive effects on uniformity of blastocysts at d12 of pregnancy in gilts. Additionally, effects of dextrose (plus lactose) in pre-mating sow diets on

litter size, piglet birth weight and uniformity were more pronounced when the treatment started already during lactation (Van den Brand et al., 2006; 2009). And Zak et al. (1997a) showed already that feed restriction during the last week of lactation dramatically reduces embryo survival at d28 of the next pregnancy in primiparous sows (64 vs. 87% compared with sows fed ad libitum during the last week of lactation). This would suggest that the effects of pre-mating diets on litter uniformity are stronger when dietary treatments start already during (late) lactation.

Results of this study show that in multiparous sows, insulin levels during the WOI are related to both plasma progesterone levels and conceptus development during early pregnancy. Mean insulin level and insulin AUC720 at d2 and 3 after weaning were positively related to both mean progesterone level during the first 10d of pregnancy and maximal progesterone level at d10 of pregnancy. Furthermore, insulin AUC720 was positively related to conceptus diameter at d10 of pregnancy.

Progesterone level and conceptus development at d10 of pregnancy were highly correlated in this study. It is questionable, however, whether this correlation is causal (i.e. higher progesterone levels advance conceptus development) or whether both the improved progesterone levels and improved conceptus development share a common origin. Plasma progesterone levels during early pregnancy are related to embryonal survival (Ashworth, 1991; Jindal et al., 1996; 1997; Van den Brand et al., 2000). Progesterone regulates quantitative and qualitative changes in uterine protein secretion (Knight et al., 1973; Stroband and Van der Lende, 1990; Davis and Blair, 1993; Vallet et al., 1998), which are essential for conceptus development. In sows, effects of progesterone on uterine secretions and embryo development are mostly studied after d10 of pregnancy (because most uterine proteins are produced after d10). Progesterone treatments at d2 and 3 of pregnancy have been shown to increase uterine total protein at d10-15 and advance conceptus estrogen production at d11 of pregnancy (Vallet et al., 1998; Vallet and Christenson, 2004), suggesting that indeed the relationship between progesterone and conceptus development could be causal. However, it remains speculative whether the natural variation in progesterone levels among sows, or the variation in progesterone levels between sows in this study, results in significant differences in uterine protein secretion during the first 10d of pregnancy among sows, and if so, whether it will result in visible changes in conceptus development at d10. Ashworth et al. (1999a; 1999b) showed that pre-mating nutrition can affect embryo survival and development (as indicated by e.g. number of cells and in vitro blastocyst CO_2 production) at d12 of pregnancy, without significant changes in plasma progesterone levels and uterine fluid composition. We did not find relationships between pre-ovulatory insulin levels and uterine secretions (insulin, calcium and acid phosphatase activity) in this study. Therefore, the correlation found between progesterone levels and conceptus development at d10 of pregnancy in the current study, may

not be (completely) causal, but might also be the result of 2 different processes sharing a common origin, that is follicle and oocyte development.

Effects of insulin levels during the WOI on subsequent progesterone secretion and conceptus development are most probably mediated through effects of insulin on follicle and oocyte development. Insulin is known to stimulate follicle and oocyte development, as discussed elsewhere (Wientjes et al., submitted). Both insulin AUC720 and mean insulin at d2 and 3 after weaning were positively related to basal LH levels around the LH surge, and basal insulin levels at d2 and 3 after weaning were positively related to follicle size at ovulation (Wientjes et al., submitted).

We found positive correlations between mean follicle diameter at d4 after weaning and both mean CL diameter and mean CL weight, indicating that larger follicles develop into larger CL's. This is further confirmed by results of Soede et al. (1998), who found a positive relationship between average follicle volume at ovulation (as measured by ultrasound) and average CL weight ($r = 0.28$; $P < 0.01$) at d5 of pregnancy in sows. More evidence for a direct relationship between pre-ovulatory follicle development and subsequent luteal development and progesterone secretion comes from studies in cows. In estrus-synchronized cows, Vasconcelos et al. (2001) artificially reduced pre-ovulatory follicle size by aspirating all follicles larger than 4 mm 5 or 6d before induction of ovulation, and showed that CL volumes at d7 and d14 of pregnancy were positively related to pre-ovulatory follicle size ($r > 0.57$; $P < 0.01$). Additionally, smaller pre-ovulatory follicles resulted in lower plasma progesterone levels during early pregnancy. In spontaneous ovulating cows, Echternkamp et al. (2009) found positive correlations between follicle size at AI (12h after detection of estrus) and both subsequent CL diameter ($r = 0.54$; $P < 0.001$) and plasma progesterone levels ($r = 0.29$; $P < 0.01$) during d7-15 after AI.

In sows, the relationship between CL size and plasma progesterone level is confounded by ovulation rate. In the current study, individual CL diameter and weight were not related to plasma progesterone levels, but total luteal weight was strongly correlated with maximal plasma progesterone level at d10 of pregnancy ($r = 0.51$; $P < 0.01$). Strong evidence for a relationship between total luteal weight and plasma progesterone levels in sows is still lacking, because most studies only used ovulation rate as a measure for luteal development. However, recently we also found a positive correlation between plasma progesterone levels and total luteal weight ($r = 0.5$; $P < 0.05$) at d5 of pregnancy (N.M. Soede, unpublished results). Therefore, we conclude that an increased total luteal weight, as a result of either a higher ovulation rate or larger individual CL's or both, is beneficial for progesterone production and secretion in sows.

Several indications exist for a relationship between pre-ovulatory follicle and oocyte development with subsequent embryo development. Pope et al. (1990) reviewed that distributions of follicular development, oocyte maturation and zygotes within sows are similarly skewed, with a majority of follicles/oocytes/zygotes being more developed than a lesser developed minority. Xie et al. (1990) demonstrated that oocytes from earlier ovulating, i.e. further-developed, follicles subsequently became the further-developed embryos at d4 and d12 of pregnancy. Additionally, pre-mating feeding strategies, shown to be beneficial for embryo development and survival, have beneficial effects on follicle size (Zak et al., 1997a; 1997b), oocyte maturation (Zak et al., 1997b; Ferguson et al., 2003; 2006; 2007), and follicular fluid composition (Zak et al., 1997b; Ferguson et al., 2003) as well. Together, these studies indicate that follicle and oocyte development (and uniformity) may be major determinants of subsequent embryo development (and uniformity).
In the present study conceptus development at d10 of pregnancy was not correlated with pre-ovulatory follicle diameter, which might indicate several things. Firstly, it might indicate that follicle size is not a good indicator for oocyte development. Although several studies reported relations between follicle size and a). intra-follicular steroid environment, as indicated by e.g. estradiol concentration and content (Biggs et al., 1993; Zak et al., 1997b); and b). (stage of) oocyte maturation, as indicated by e.g. in vitro maturation rates (Hunter and Wiesak, 1990; Vatzias and Hagen, 1999), Hunter & Wiesak (1990) also reported that follicles of similar size can show marked differences in follicular fluid composition. This is confirmed by, for example, results of Ferguson et al. (2003; 2007), who reported positive effects of a high feeding level or high fiber diets prior to mating on oocyte maturation and follicular fluid composition, without effects on follicle diameter. Therefore, it remains speculative whether follicle diameter in the current study was indicative for oocyte development. Secondly, it is possible that differences in oocyte development are not visible in d10 conceptuses. The rapid development of conceptuses around this stage (within 24h conceptuses change from spherical to filamentous), makes conceptus development at d10 of pregnancy a highly variable trait. But also factors other than oocyte maturation (e.g. uterine secretions) may have influenced d10 conceptus development.

Our results suggest that high insulin levels during the WOI are beneficial for the subsequent pregnancy in sows, both for progesterone secretion and for conceptus development. Beneficial effects of a high feeding level (2.6 x maintenance) or a high fibre diet (50% sugarbeet pulp) during the estrous cycle prior to mating on subsequent embryo development and embryo survival in gilts have been reported previously (Ashworth et al., 1999b; Ferguson et al., 2006; 2007). Although insulin profiles were not measured in those studies, effects might have been mediated through enhanced pre-mating insulin levels as a result of the high feeding level (e.g. as shown by Booth et al. (1996) and Quesnel et al. (1998)) and the high fibre diet (e.g. as shown by Vestergaard (1997)). Additionally, Ashworth et al. (1999a) reported heavier CL’s at

d12 of pregnancy in gilts fed a high pre-mating diet compared to gilts fed a maintenance pre-mating diet. Kemp et al. (1995) found higher plasma progesterone levels during early pregnancy in sows fed a starch-rich diet compared with sows fed a fat-rich diet during lactation, WEI and gestation, which might be related to the insulin-stimulating effect of starch-rich diets (as shown by e.g. Van den Brand et al. (1998)).
Together, these data support our finding that pre-ovulatory insulin levels can influence both subsequent luteal development and progesterone secretion, as well as embryo development. These effects are most probably mediated through beneficial effects of insulin on both follicle and oocyte development.

IGF-1 levels at d3-5 after weaning were not related to luteal development, progesterone levels and conceptus development and uniformity in this study. This could indicate that IGF-1 levels during WOI do not play a major role in subsequent embryo development and uniformity in multiparous sows.

The CTRL diet fed at 12h intervals resulted in larger CL's (related with larger pre-ovulatory follicles) and further developed conceptuses compared with the DL diet fed at 4h intervals. These beneficial effects of the CTRL treatment might be related to the secretion pattern of insulin (2 long insulin peaks/day vs. 6 short insulin peaks/day), and suggests that not only the total amount of insulin secreted, but also the pattern of insulin secretion during the day can affect follicle development and subsequent luteal and conceptus development (see further Wientjes et al., submitted).

IMPLICATIONS

High insulin levels during the WOI are beneficial for subsequent pregnancy in multiparous sows, as indicated by positive relationships between pre-ovulatory insulin levels and plasma progesterone levels and conceptus development during the first 10d of pregnancy. Those effects are most probably mediated through beneficial effects of insulin on follicle and oocyte development, which is reflected in both improved luteal development and improved conceptus development.
Insulin profiles during the WOI were not related to uniformity of pre-implantation conceptuses, but improved progesterone levels and improved conceptus development as a result of enhanced insulin levels during the WOI might be beneficial for survival and uniformity of embryos and fetuses at later stages of pregnancy.

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Table 1. Luteal development and progesterone characteristics (means ± SE) for sows fed either a dextrose- and lactose (each 150 g/d) containing diet (DL) at 4h intervals or an isocaloric control diet (CTRL) at 12h intervals during the WOI (from weaning until 12h after ovulation)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	DMI_{WOI}[2]
Number of sows	15	16[3]		
<u>Luteal development</u>				
Ovulation rate	24.3 ± 1.2	23.2 ± 0.8	0.43	-
Total luteal weight[4], g	11.2 ± 0.5	9.7 ± 0.5	0.03	-
Mean CL diameter[4], mm	10.0 ± 0.3	9.6 ± 0.3	0.06	< 0.01[6]
SD[4], mm	0.7 ± 0.06	0.8 ± 0.07	0.33	-
CV[4], %	7.5 ± 0.7	8.7 ± 0.7	0.15	-
Mean CL weight[4], g	0.47 ± 0.02	0.42 ± 0.02	0.09	-
SD[4], g	0.05 ± 0.01	0.05 ± 0.00	0.82	-
CV[4], %	11.5 ± 1.4	12.2 ± 0.5	0.60	-
<u>Progesterone</u>				
Basal progesterone, ng/ml[5]	0.49 ± 0.07	0.86 ± 0.21	0.23	0.06[6]
Mean progesterone, ng/ml[5]	14.60 ± 1.35	14.70 ± 0.90	0.96	0.05[6]
Maximal progesterone, ng/ml	30.57 ± 2.14	29.71 ± 1.91	0.28	-

[1] Statistical significance; the treatment*DMI_{WOI} interactions were never significant; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).

[2] DMI from weaning until 12h after ovulation, as % of total dry matter offered (< 75%, ≥ 75%).

[3] Additionally, 1 DL sow developed cystic ovaries.

[4] Additionally, 2 CTRL sows had cystic CL's on both ovaries.

[5] Only for sows with a jugular vein catheter (n = 10 CTRL and 11 DL sows).

[6] For sows with a low (< 75%) and high (≥ 75%) DMI respectively, LS means were 10.4 and 9.4 mm for mean CL diameter, 0.89 and 0.45 ng/ml for basal progesterone, and 13.60 and 16.31 ng/ml for mean progesterone.

Table 2. Conceptus development and uniformity at d10 of pregnancy (means ± SE) for sows fed either a dextrose- and lactose (each 150 g/d) containing diet (DL) at 4h intervals or an isocaloric control diet (CTRL) at 12h intervals during the WOI (from weaning until 12h after ovulation)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	DMI_{WOI} [2]
Number of pregnant sows	14[3]	14[3]		
Number of conceptuses[4]	21.9 ± 1.1	20.2 ± 0.9	0.27	-
Embryonal survival[4], %	90 ± 2	88 ± 2	0.99	-
Diameter[4,5,6], mm	7.1 ± 0.47	6.4 ± 0.64	0.07	-
SD[4,5,6], mm	1.2 ± 0.14	1.2 ± 0.14	0.65	-
CV[4,5], %	17.4 ± 1.64	18.8 ± 2.14	0.60	-
Surface area[4,5,6], mm^2	73.0 ± 10.5	63.4 ± 11.2	0.11	-
SD[4,5,6], mm^2	20.3 ± 2.5	19.0 ± 3.0	0.20	-
CV[4,5], %	28.6 ± 1.9	31.4 ± 2.8	0.40	-
Embryoblast diameter[4,5,6], mm	0.43 ± 0.02	0.40 ± 0.03	0.20	-
SD[4,5,6], mm	0.07 ± 0.01	0.07 ± 0.01	0.77	-
CV[4,5], %	17.5 ± 1.2	18.2 ± 1.5	0.72	-
Protein content[4,5,6], µg	86 ± 9	75 ± 11	0.29	-
SD[4,5,6], µg	26 ± 2	23 ± 3	0.23	-
CV[4,5], %	32 ± 2	32 ± 3	0.88	-
DNA content[4,5,6], ng	349 ± 33	329 ± 36	0.32	-
SD[4,5,6], ng	169 ± 14	138 ± 15	0.08	-
CV[4,5], %	44 ± 9	46 ± 5	0.88	-

[1] Statistical significance; the treatment*DMI interactions were never significant; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).

[2] DMI from weaning until 12h after ovulation, as % of total dry matter offered (< 75%, ≥ 75%).

[3] Additionally, 1 DL sow had silent estrus, 1 DL sow developed cystic ovaries and in 2 sows (1 CTRL and 1 DL) no conceptuses were recovered.

[4] 1 sow (DL) with non-vital conceptuses was excluded.

[5] 2 sows (1 CTRL and 1 DL) with filamentous conceptuses were excluded.

[6] Corrected for significant effect ($P < 0.05$) of age of conceptuses (9.5d or 10d).

Table 3. Relations ($P < 0.05$) between insulin parameters (mean values of d2 and 3 after weaning) and luteal development and progesterone parameters, and conceptus development and uniformity parameters

	Insulin parameters[1]	
Luteal development and progesterone	Insulin AUC720, μU	Mean insulin, μU/ml
Mean CL diameter, mm	n.s.	ß = - 0.06 mm / (μU/ml) $P = 0.01$
Mean progesterone, ng/ml	ß = 0.35 (ng/ml) / 1,000 μU $P = 0.02$	ß = 0.14 (ng/ml) / (μU/ml) $P = 0.05$
Maximal progesterone, ng/ml	ß = 0.73 (ng/ml) / 1,000 μU $P < 0.01$	ß = 0.27 (ng/ml) / (μU/ml) $P = 0.05$
Conceptus development and uniformity		
Conceptus diameter, mm	ß = 0.15 mm / 1,000 μU $P = 0.03$	n.s.
SD of conceptus surface area, mm^2	ß = 0.61 mm^2 / 1,000 μU $P = 0.05$	n.s.

Overall treatment-corrected regressions ($P < 0.05$) are presented; when significant batch- and/or conceptus-age-effects existed ($P < 0.10$), regressions were corrected for these effects.
[1] Mean value of d2 and 3 after weaning.

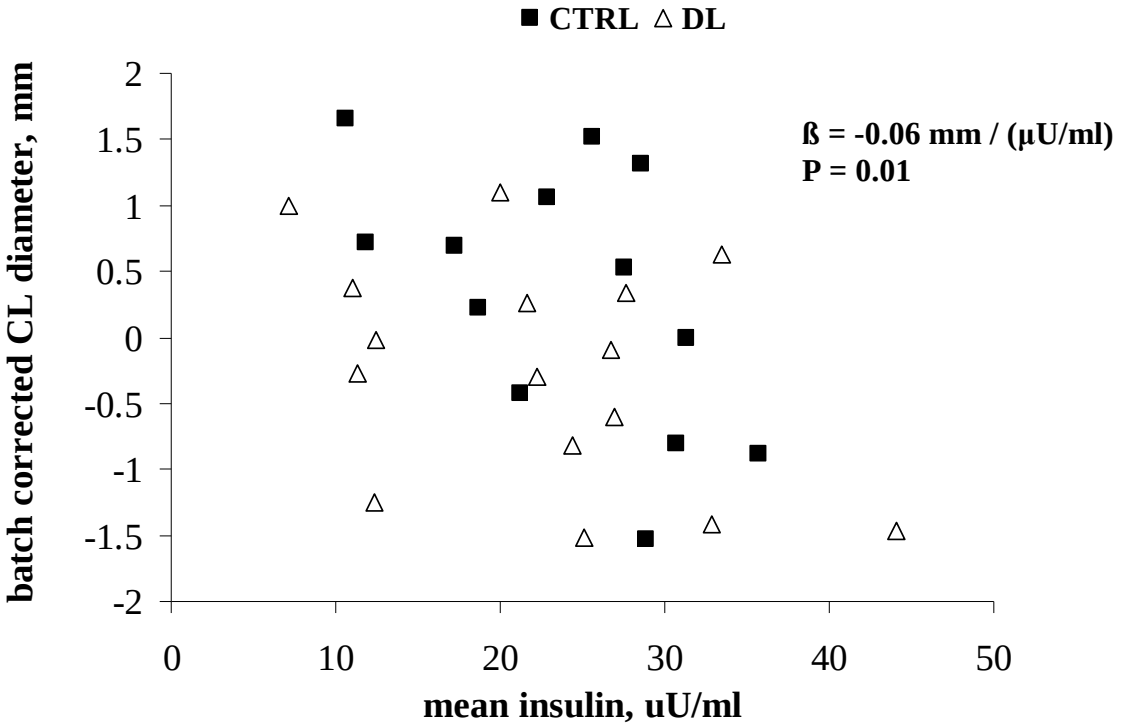

Figure 1. Relation between mean insulin level (mean d2 and 3 after weaning) and CL diameter (residuals corrected for the effect of batch; LSmeans were 10.5, 9.6 and 9.6 mm for batch 1, 2 and 3 respectively; *P* = 0.04; CL diameter tended to be higher in CTRL compared with DL; 10.0 vs. 9.6 mm; *P* = 0.06)

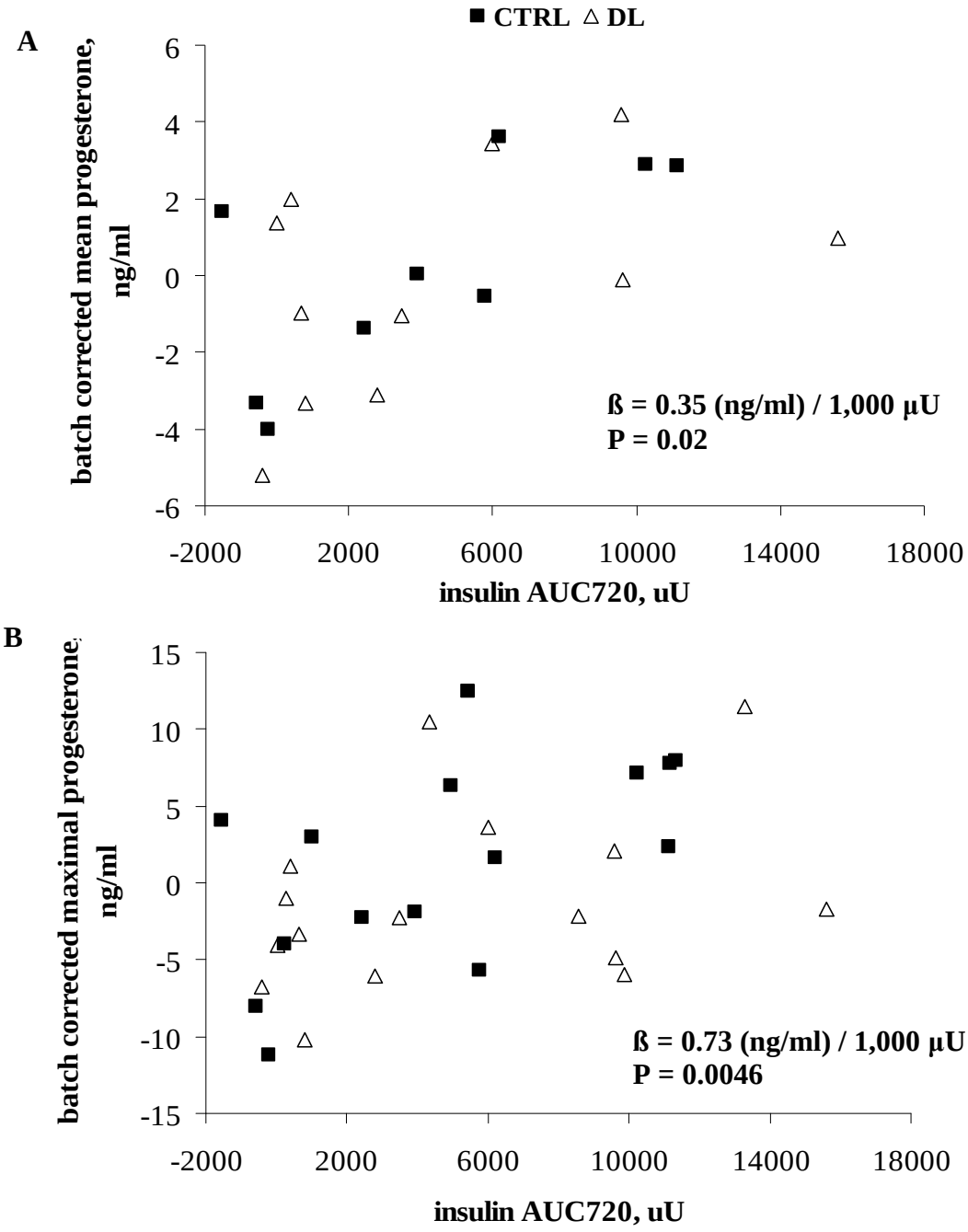

Figure 2. Relations between insulin AUC720 (mean d2 and 3 after weaning) and A). mean progesterone level (residuals corrected for the effect of batch; LSmeans were 18.14, 13.92 and 12.80 ng/ml for batch 1, 2 and 3 respectively; $P < 0.01$); and B). maximal progesterone level at d10 of pregnancy (residuals corrected for the effect of batch; LSmeans were 37.47, 28.84 and 26.04 ng/ml for batch 1, 2 and 3 respectively; $P < 0.01$)

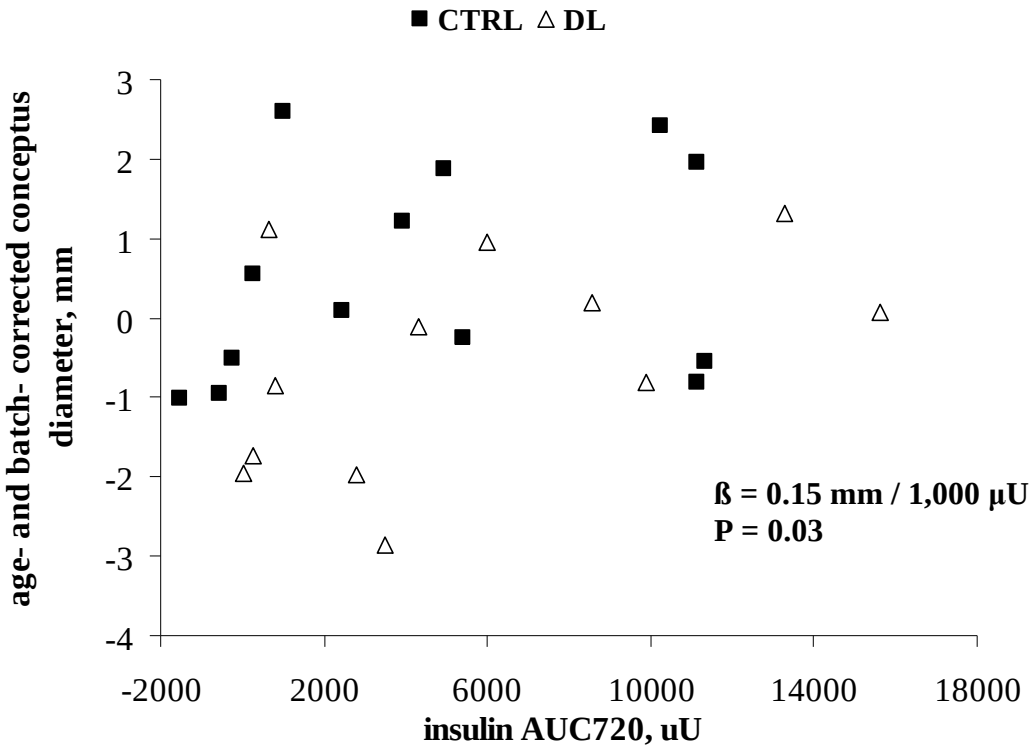

Figure 3. Relation between insulin AUC720 (mean d2 and 3 after weaning) and conceptus diameter (residuals corrected for the effect of batch and conceptus-age; LSmeans were 7.9, 5.9 and 6.0 for batch 1, 2 and 3, respectively ($P = 0.04$); LSmeans were 5.4 and 7.7 for 9.5 and 10d-old conceptuses, respectively ($P < 0.01$; conceptus diameter tended to be higher in CTRL compared with DL; 7.1 vs. 6.4 mm; $P = 0.07$)

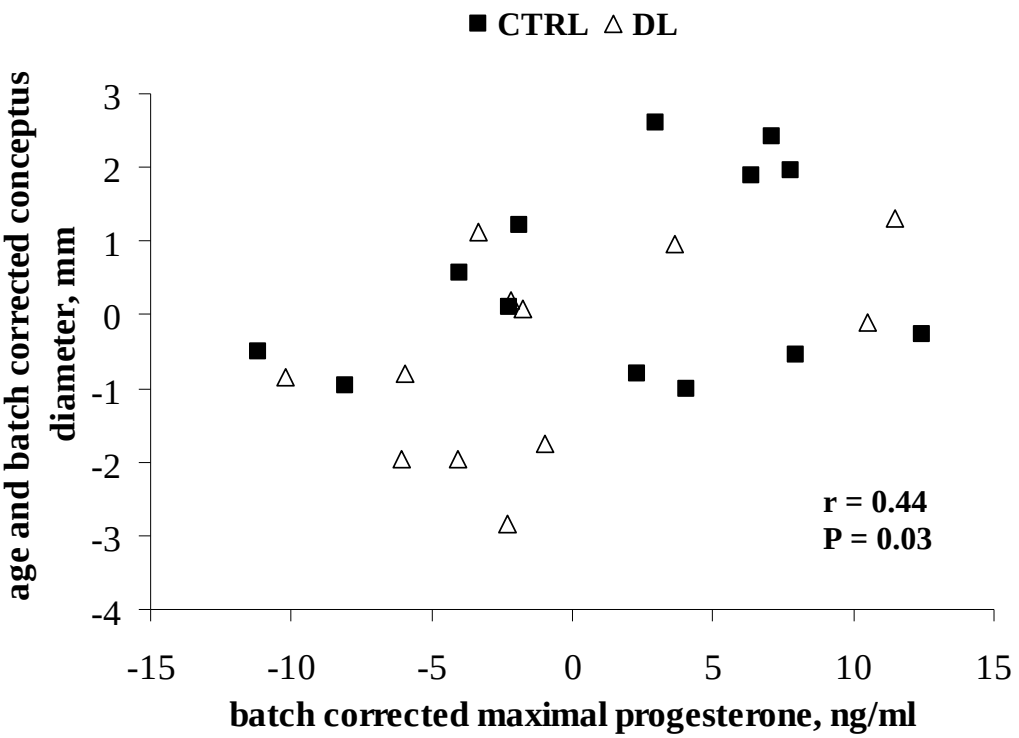

Figure 4. Correlation between maximal progesterone level at d10 of pregnancy (residuals corrected for the effect of batch; LSmeans were 37.47, 28.84 and 26.04 ng/ml for batch 1, 2 and 3 respectively; $P < 0.01$) and conceptus diameter (residuals corrected for the effect of batch and conceptus-age; LSmeans were 7.9, 5.9 and 6.0 for batch 1, 2 and 3, respectively ($P = 0.04$); LSmeans were 5.4 and 7.7 for 9.5 and 10d-old conceptuses, respectively ($P < 0.01$)